European Journal of Pharmacology 816 (2017) 47–57

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

NKT cells in cardiovascular diseases

Gijs H.M. van Puijvelde*, Johan Kuiper

Division of Biopharmaceutics, Leiden Academic Centre for Drug Research, Leiden University, Einsteinweg 55, 2333CC Leiden, The Netherlands

ARTICLE INFO

Keywords:
Cardiovascular diseases
Atherosclerosis
Inflammation
NKT cells

ABSTRACT

Despite life-style advice and the prescription of cholesterol-lowering and anti-thrombotic drugs, cardiovascular diseases are still the leading cause of death worldwide. Therefore, there is an urgent need for new therapeutic strategies focussing on atherosclerosis, the major underlying pathology of cardiovascular diseases characterized by an accumulation of lipids in an inflamed arterial/vessel wall. CD1d-restricted lipid-sensing natural killer T (NKT) cells, bridging the innate and adaptive immunity, and CD1d-expressing antigen-presenting cells are detected in atherosclerotic lesions of mice and humans. In this review we will summarize studies that point to a critical role for NKT cells in the pathogenesis of atherosclerosis and other cardiovascular diseases by the secretion of pro-atherogenic cytokines and cytotoxins. These pro-atherogenic NKT cells are potential targets for new therapeutic strategies in the prevention and treatment of cardiovascular diseases. Additionally, proteins transferring lipids during atherosclerosis, which are also important in the loading of lipids onto CD1d and possible endogenous ligands responsible for the activation of NKT cells during atherosclerosis will be discussed.

1. Introduction

It is well established that both innate and adaptive immunity play a significant role in cardiovascular diseases, including the development and progression of atherosclerosis which was traditionally seen as a lipid-disorder (Hansson, 2001). The immune responses are mainly triggered by the accumulation and modification of low-density lipoproteins (LDL) in the vessel wall. LDL particles, rich in cholesterol, get oxidized or glycated, aggregate, associate with proteoglycans or are incorporated into immune complexes (Khoo et al., 1992, 1988) resulting in immunogenic particles. Macrophages take up the modified and especially oxidized LDL (oxLDL) via scavenger receptors leading to the formation of lipid-loaded foam cells and the secretion of cytokines and chemokines. This is one of the key-processes in the development of atherosclerosis (Han et al., 1997; Khoo et al., 1992). Subsequently, immunogenic peptide and lipid antigens, especially derived from modified auto-antigens such as oxLDL and heat shock proteins (i.e. HSP-60/65), are presented by surface CD1 (lipid antigens) or major histocompatibility complex (MHC) class I and II molecules (peptide antigens) on macrophages, dendritic cells (DCs) and B cells. This initiates the activation of adaptive immune cells, primarily $CD4^+$ T cells including oxLDL-specific (Hansson et al., 1989; Stemme et al., 1995) and HSP60/65-specific T cells (Afek et al., 2000; Benagiano et al., 2005). Upon recognition of the peptides the naïve T cells turn into effector (memory) T cells, especially Th1 effector cells, producing the pro-atherogenic cytokine IFN-γ. The local production of cytokines and chemokines by inflammatory cells, leads to a continuous influx of inflammatory cells into the vessel wall and together with an ineffective clearing of dead cells (efferocytosis) this results in a chronic inflammatory process, progressing atherosclerotic lesion development.

Since lipids are very important in the development of atherosclerosis, the discovery of lipid-sensing T cells, the natural killer T (NKT) cells, led to an intensive investigation on the possible role of these cells in cardiovascular diseases and especially in atherosclerosis. Here we review the role and function of NKT cells in cardiovascular diseases with a special focus on atherosclerosis and the possibilities to target or modulate these cells as a therapeutic strategy in the prevention of cardiovascular diseases.

2. NKT cells

NKT cells represent a distinct subset of T cells expressing unique TCRs and surface markers commonly expressed by NK cells including NK1.1, Ly49, CD16 and CD122. The unique TCRs of NKT cells are restricted to certain (glyco)lipid antigens while conventional T cells show a wide diversity of TCRs directed against numerous peptide antigens. The (glyco)lipids are presented to the NKT cells on the MHC class I like molecule CD1d in association with β_2-microglobulin. CD1d, highly conserved in mammalian species, is constitutively expressed on antigen-presenting cells (APCs) such as macrophages, DCs and B cells. In addition, CD1d is also expressed by other cells including thymocytes necessary for the development of NKT cells (Gapin et al., 2001), hepatocytes (Agrati et al., 2005), intestinal Paneth cells (Monzon-Casanova et al., 2010) and vascular smooth muscle cells (VSMCs) (Canchis et al., 1993). The CD1d-restricted

* Corresponding author.
E-mail address: puijvelde@lacdr.leidenuniv.nl (G.H.M. van Puijvelde).

http://dx.doi.org/10.1016/j.ejphar.2017.03.052
Received 9 December 2016; Received in revised form 10 March 2017; Accepted 23 March 2017
Available online 29 March 2017

NKT cells can be subdivided into two types. Type I NKT cells, also referred to as invariant (i)NKT cells are the most common ones in mice expressing a semi-invariant T cell receptor (TCR) composed of a Vα14-Jα18 (formerly Vα14-Jα281) TCRα chain paired with a Vβ2, Vβ7 or Vβ8.2 TCRβ chain, while in humans this TCR is composed of a Vα24Jα18 TCRα chain and a Vβ11 TCRβ chain. Type II NKT cells, the most common NKT cell type in humans but less frequent in mice, do not express these invariant TCRs but have more variable TCRs. In mice type I NKT cells can again be subdivided into two subsets distinguishable by the expression of CD4 and CD8: $CD4^+CD8^-$ NKT cells and CD4/CD8 double negative (DN) NKT cells. Humans have an additional subset of $CD4^-CD8^+$ NKT cells. Type I and Type II NKT cells can also be distinguished based upon lipid recognition. Type I NKT cells are potently activated by α-galactosylceramide (α-GalCer), while type II NKT cells respond to the self-glycolipid sulfatide, present in several membranes throughout the body (Arrenberg et al., 2010; Jahng et al., 2004). In mice, NKT cells are most frequently found in the liver (10–35% of liver lymphocyte population) and less frequent (0.2–2.5% of lymphocytes) in spleen, thymus, lymph nodes, bone marrow and blood. In humans, the percentage of NKT cells within the lymphocyte population throughout the body is much lower ranging from 0.3% to 1%.

NKT cells can get activated via different pathways including CD1d/TCR-dependent pathways, discussed in more detail in Sections 4–6, but also CD1d/TCR-independent pathways such as cytokine-driven activation. Cytokines, such as IL-12 and IL-18, produced by pathogen-activated APCs during for example infections with *Salmonella typhimurium, Staphylococcus aureus* and *Mycobacterium tuberculosis* can activate the NKT cells via the IL-12R and IL-18R expressed on their surface (Eberl and MacDonald, 1998; Leite-De-Moraes et al., 1999). More recent studies have shown that IL-33 in cooperation with IL-12 can activate and stimulate IFN-γ production by NKT cells (Bourgeois et al., 2009). In general it is a combination of both CD1d-dependent and CD1d-independent pathways leading to the activation of NKT cells. Although NKT cells might have a lower threshold for activation than conventional T cells, co-stimulatory pathways such as CD28/B7, ICOS/ICOSL, CD40L/CD40, and OX40/OX40L and co-inhibitory pathways such as PD1/PD-L also influence the activation status of NKT cells.

Upon stimulation, all NKT cells react rapidly by secreting robust amounts of a variety of pro-inflammatory Th1 cytokines (IFN-γ, IL-12, TNF-α, IL-2, IL-6) and anti-inflammatory Th2 cytokines (IL-4, IL-5, IL-10, IL-13). Additionally, NKT cells rapidly acquire cytolitic activity which results in the secretion of granzyme B, perforin, or FasL. This makes NKT cells a unique cell type bridging the adaptive and the innate immune system. Activation of NKT cells also leads to a robust bystander activation of a wide variety of other inflammatory cells such as dendritic cells (Fujii et al., 2003; Kitamura et al., 1999), monocytes (Hegde et al., 2009), macrophages (Villanueva et al., 2015), NK cells (Carnaud et al., 1999), B cells (Kitamura et al., 2000) and T cells (Eberl et al., 2000; Nishimura et al., 2000).

Numerous studies have shown that dysfunctional NKT cells or reduced numbers of NKT cells in humans and mice can be linked to several diseases varying from cancer (Tahir et al., 2001) to infectious (Kee et al., 2012; Mureithi et al., 2011) and autoimmune diseases (Kis et al., 2007; van der Vliet et al., 2001; Yanagihara et al., 1999). This is confirmed by a bunch of studies with mouse models showing that an adoptive transfer of NKT cells or glycolipid-induced activation of NKT cells (mostly α-GalCer, discussed in Sections 4 and 5) leads to a protection against diabetes (Hong et al., 2001), experimental autoimmune encephalomyelitis (Jahng et al., 2001), colitis (Saubermann et al., 2000), arthritis (Coppieters et al., 2007) and several types of cancer (Van Kaer et al., 2011).

3. NKT cells in atherosclerosis

3.1. CD1d-expressing cells and NKT cells in atherosclerotic lesions

The first study on a possible role of NKT cells in atherosclerosis emerged in 2002. In this study $apoE^{-/-}$ mice were treated with LPS and showed increased plaque size correlating with increased numbers of IL-4 producing NKT cells. It was suggested that these NKT cells might be responsible for the increased levels of autoantibodies against oxLDL leading to the increase in atherosclerotic lesion development (Ostos et al., 2002). Bobryshev et al. and Melián et al. were the first to detect the expression of CD1d on respectively DCs and macrophages in human atherosclerotic lesions (Bobryshev and Lord, 2002; Meliá et al., 1999). Later on, NKT cells were detected in human atherosclerotic lesions isolated from aneurysm patients. NKT cells expressing the IL-18 receptor predominated over NKT cells expressing ST2L and it was suggested that the IL-18R and ST2L expression on NKT cells might be an important determinant of the immune status in atherosclerosis (Chan et al., 2003). Bobryshev et al. established that NKT cells, present in human carotid arteries with advanced atherosclerotic lesions co-localize with CD1d expressing DCs in the plaque shoulders of the lesion. The direct interaction between these cells confirmed that NKT cell activation occurs inside the lesion and may contribute to plaque instability (Bobryshev and Lord, 2005).

3.2. CD1d deficiency and atherosclerosis

These studies were the beginning of an increased interest in the possible role of NKT cells during hyperlipidaemia and atherosclerosis, especially because of the lipid-sensing capacity of these cells and their association with decreasing the inflammatory response. However, atherosclerotic lesion formation at the aortic root was found to be reduced in $CD1d^{-/-}$ mice, lacking type I and II NKT cells, when compared with wild-type (C57Bl6) mice and in $CD1d^{-/-}$→$LDLr^{-/-}$ chimeras when compared with WT→$LDLr^{-/-}$ chimeras all fed a high-fat diet containing fat, cholesterol and cholic acid (Nakai et al., 2004). Subsequently, $ApoE^{-/-}$ and $LDLr^{-/-}$ mice were backcrossed with $CD1d^{-/-}$ mice to generate hyperlipidemic mice lacking functional type I and type II NKT cells. The deficiency in CD1d led to a reduced atherosclerotic lesion size in both the aortic root and throughout the whole aorta of mice fed a regular chow diet (Major et al., 2004; Tupin et al., 2004) or a Western type diet (Aslanian et al., 2005), showing that NKT cells have a pro-atherogenic function. The effects on lesion size were especially seen during the initial stage of atherosclerosis characterized by mild hypercholesterolemia and fatty streak formation which is consistent with the suggestion that NKT cells mediate early immune responses at inflammatory sites before the conventional T cells are activated. Additionally, Ström et al. showed a reduced intima thickening in the carotids of $CD1d^{-/-}$ mice upon vascular injury induced by a perivascular collar around the carotid arteries under normocholesterolemic conditions (Ström et al., 2007).

3.3. NKT cell subsets and atherosclerosis

To determine whether type I $Vα14^+$ iNKT cells are important in early atherosclerosis, $LDLr^{-/-}Jα281^{-/-}$ mice which lack iNKT cells were generated and fed a high fat diet. Compared with $LDLr^{-/-}$ mice atherosclerotic lesion size was again reduced, establishing the profound role of iNKT cells in atherosclerosis. In addition, $LDLr^{-/-}Jα281^{-/-}$ mice showed reduced splenocyte-secreted and lesion-associated IFN-γ concentrations providing strong evidence that the iNKT cells promote atherosclerosis by producing IFN-γ themselves or by stimulating the secretion of IFN-γ by other cells (Rogers et al., 2008). In accordance, VanderLaan et al. showed that a transfer of splenocytes enriched with type I $Vα14^+$ iNKT cells ($Vα14^{tg}$) to $RAG1^{-/-}LDLr^{-/-}$ mice increased atherosclerotic lesion size, and Subramanian et al. showed a similar increase in lesion size in $LDLr^{-/-}$ mice crossed with $Vα14^{tg}$ mice. Both studies demonstrate that the activation of the pro-atherogenic iNKT cells relies on endogenous ligands, since no exogenous NKT cell antigens are administered (Subramanian et al., 2013; VanderLaan et al., 2007). A comparative study including both $LDLr^{-/-}Jα281^{-/-}$ mice

and LDLr$^{-/-}$CD1d$^{-/-}$ mice fed the same high fat diet should now be performed to give more insight in a possible contribution of other types of CD1d-dependent NKT cells i.e. type II NKT cells.

In a more advanced approach to determine the contribution of different subtypes of NKT cells to atherosclerosis, an adoptive transfer of NKT cells to apoE$^{-/-}$ mice depleted of NKT cells by thymectomy showed that CD4$^+$ NKT cells but not DN (CD4$^-$CD8$^-$) NKT cells augment the development of atherosclerosis (To et al., 2009). Similar to the study by VanderLaan et al. (2007) this study suggests that NKT cells are activated in vivo by endogenous ligands since no exogenous ligands were administered to the thymectomized mice. The difference in the pro-atherogenic capacity of both subtypes is associated with the differential expression of Ly49, an inhibitory receptor which can be activated by MHC class I molecules. Ly49 is more abundantly expressed on the DN NKT cells and the high expression of MHC class I in atherosclerotic lesions may therefore lead to a less pronounced role of these highly pro-inflammatory cells in atherosclerosis. Although a transfer of DN NKT cells did not have an effect on lesion size, they exhibited an equal ability as CD4$^+$ NKT cells to reduce the collagen content of atherosclerotic lesions which proves that these effects are independent of Ly49 and inflammatory cytokines such as IFN-γ (To et al., 2009). The effect of NKT cells on collagen content of lesions, already shown by Nakai et al. (2004) establishes the differential effects of NKT cells in different stages of atherosclerosis. Although CD1d$^+$ cells are found more frequently in advanced atherosclerotic lesions than in early lesions (Kyriakakis et al., 2010), no differences in lesion size were observed upon NKT cell deficiency in more advanced stages of atherosclerosis in mice (Aslanian et al., 2005). However, NKT cells might still play a role in these more advanced stages by affecting the collagen content and subsequently the lesion stability.

3.4. How NKT cells contribute to atherosclerotic lesion development

Until 2014, it was unknown how NKT cells exactly contribute to the development of atherosclerotic lesions. As shown in Fig. 1, this could either be via the increased secretion of pro-inflammatory and pro-atherogenic cytokines by NKT cells or bystander activated leukocytes, or by the induction of apoptosis via the cytotoxins of NKT cells, granzyme B, perforin and FasL. Li et al. (2015) showed that adoptive transfer of liver NKT cells into T- and B-cell deficient apoE$^{-/-}$Rag2$^{-/-}$ mice and T-, B- and NK cell deficient apoE$^{-/-}$Rag2$^{-/-}$γc$^{-/-}$ mice still augmented atherosclerotic lesion development indicating that neither T and B cells nor NK cells are necessary for NKT cells to affect the development of atherosclerosis. These results disprove the study by Andoh et al. in which they suggested that LPS-induced NKT cell activation leads to an increase in and activation of NK cells, possibly induced by NKT cell derived IFN-γ, and that these NK cells further promote atherosclerotic lesion development (Andoh et al., 2013). This link between NKT and NK cells can still be important, but is not requisite for the contribution of NKT cells to lesion development.

Although it is obvious that cytokines, especially IFN-γ, produced by NKT cells influence the development of atherosclerotic lesions, Li et al. showed that CD4$^+$ NKT cells can affect lesion development in a cytokine-independent way, since IFN-γ$^{-/-}$, IL-4$^{-/-}$ and IL-21$^{-/-}$ CD4$^+$ NKT cells were still able to increase atherosclerotic lesion size after adoptive transfer. This conclusion might be a bit premature since Ito et al. suggested that NKT cells might be pro-atherogenic via the production of TNF-α induced by the bioactive lipid sphingosine-1-phosphate (S1P). Therefore, an adoptive transfer of TNF-α deficient NKT cells should be performed (Ito et al., 2014). However, adoptive transfer of CD4$^+$ NKT cells deficient in granzyme B or perforin failed to increase atherosclerotic lesion size indicating that both perforin and granzyme B are required for the pro-atherogenic action of CD4$^+$ NKT cells. Granzyme B and perforin, known for their pro-atherosclerotic effects (Choy et al., 2003; Hiebert et al., 2013; Kyaw et al., 2013), induce apoptosis in a caspase-3 dependent way and subsequently augment post-apoptotic necrosis, appreciated by an increased necrotic core size within the lesions (Li et al., 2015). CD1d-expressing cells are specifically targeted by NKT cells for killing via the Granzyme-B/perforin mechanism (Gansuvd et al., 2002; Metelitsa et al., 2003) which means that especially CD1d expressing DCs, macrophages and thus foam cells are targets for these cells leading to more vulnerable, rupture-prone lesions with excessive apoptosis and large necrotic cores.

3.5. Prevalence of NKT cells during atherosclerosis

Studies on the prevalence of NKT cells in different organs during atherosclerosis show contradictory results. Some studies found decreased NKT cell numbers in liver and spleen of apoE$^{-/-}$ mice upon age (Major et al., 2004) or upon high fat diet feeding (Nakai et al., 2004) suggesting a decline in NKT cells upon the progression of atherosclerosis which is in line with the findings in many other autoimmune disorders (Kis et al., 2007; van der Vliet et al., 2001; Yanagihara et al., 1999). Although less frequent, the remaining NKT cells produce relatively more cytokines. The reason for the decrease in NKT cell numbers remains however elusive. Increased cell death, a down-regulation of NK1.1 and the TCR or an increased migration to peripheral tissues might be responsible for the lowering in NKT cell numbers. Since NKT cells express a broad range of chemokine receptors similar to Th1 cells such as CCR4, CCR5, CCR6, CXCR3, CXCR4 and CXCR6 (Thomas et al., 2003), it is very well possible that NKT cells are activated elsewhere in the body (liver/spleen) and then migrate to the atherosclerotic lesions upon chemokine release inside the lesions including CCL2, CCL3, CCL4, CCL5, CXCL10, CXCL12 and CXCL16, leading to the reduced NKT cell numbers in the peripheral organs. Aslanian et al. observed that NKT cells are already present in aortic arches of chow fed LDLr$^{-/-}$ mice but not in LDLr$^{+/+}$ mice. Feeding the LDLr$^{-/-}$ mice a high fat diet did not further increase the number of NKT cells which shows that a slight increase in serum cholesterol may already be adequate to induce the secretion of NKT cell recruiting chemokines (Aslanian et al., 2005). Similar to this study, Rombouts et al. (2016) detected Vα14-Jα18 mRNA in early lesions but no accumulation of Vα14-Jα18 in more advanced lesions. Further research is needed to confirm that an increased recruitment is responsible for the decline in peripheral NKT cells, especially because of the high numbers of NKT cells in the liver and spleen, and the relatively low numbers inside the lesions. On the other hand we and others found an increase in the number of NKT cells upon high fat diet feeding. NKT cell infiltration was enhanced in adipose tissue of mice on a high fat diet (Ohmura et al., 2009) and we observed an extensive increase in CD3$^+$NK1.1$^+$ NKT cell percentages in liver and spleen of LDLr$^{-/-}$ mice, but not in apoE$^{/-}$ mice both fed a high fat diet for 1.5 weeks (van Puijvelde et al., 2009).

4. Modulation of NKT cells responses during atherosclerosis

4.1. Pro-atherogenic effects of α-GalCer

As mentioned before, a glycolipid-induced activation of NKT cells leads to a protection against several autoimmune diseases. Therefore, glycolipid-induced activation of NKT cells had the potential for a possible therapy in atherosclerosis. α-GalCer is known as the most potent activator of iNKT cells and was discovered by Kirin Brewery Co., Ltd., during their search for natural anticancer medicines (Kawano et al., 1997). α-GalCer, a natural product found in the marine sponge *Agelas mauritianus* and now linked to *Sphingomonas*, bacteria which are present in the sponge, is now chemically synthesized and also known as KRN7000 (Morita et al., 1995). Especially when injected multiple times, α-GalCer has shown a great potential to dampen a wide range of immune responses in mouse models including several chronic autoimmune diseases, but also affects antitumor responses, defence

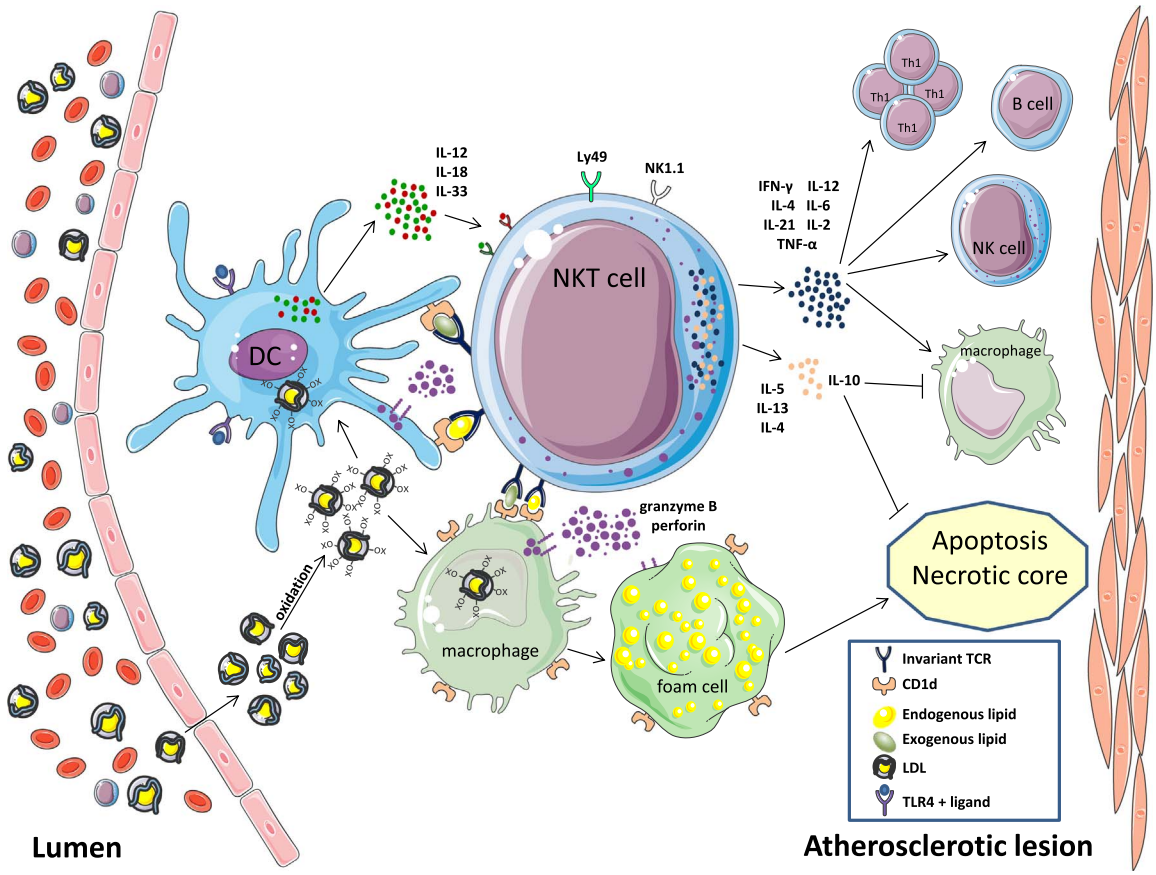

Fig. 1. How NKT cells can contribute to the development of atherosclerotic lesions. Accumulation and the subsequent modification (oxidation of LDL) of lipids in the vessel wall initiate both adaptive and innate immune responses. Macrophages take up the oxidized LDL particles and turn into lipid loaded foam cells producing cytokines and chemokines which cause the attraction of other leukocytes into the newly formed intimal layer of the vessel wall. Among these leukocytes are NKT cells which are found in close proximity of macrophages and dendritic cells that express the antigen presenting molecule CD1d. Lipids presented on CD1d may be of endogenous or exogenous origin and are recognized by the T cell receptor of the NKT cells. Although there are some possible candidates the lipid causing the activation of NKT cells during atherosclerosis is still unknown. The lipid-dependent activation of NKT cells in the atherosclerotic lesion might result in the secretion of both pro-atherogenic cytokines (IFN-γ, IL-12, IL-6, IL-21, IL-2, TNF-α and IL-4) and anti-atherogenic cytokines (IL-10, IL-4, IL-5 and IL-13). Pro-atherogenic cytokines, which are more abundantly produced than the anti-atherogenic cytokines, may cause (bystander) activation and expansion of other leukocytes such as Th1 cells, B cells, NK cells and macrophages all stimulating plaque growth. Anti-atherogenic cytokines might on their turn prevent this by inhibiting macrophages and T cell responses or by reducing the formation of necrotic cores. Lipid-independent activation of NKT cells, for example by cytokines released by Toll-like receptor 4 activated dendritic cells, might lead to the release of granzyme B and perforin which results in a caspase-3 dependent killing of CD1d expressing cells (macrophages and dendritic cells). This process contributes to the necrotic core formation and leads to bigger and especially more vulnerable lesions.

against pathogens and allergy. However, in line with the findings in NKT cell deficient mouse models, several studies with different administration regimens of α-GalCer to apoE$^{-/-}$ mice (three times in total to twice weekly over a period of 5–11 weeks) led to a CD1d-dependent increase in early atherosclerosis at the aortic root without affecting cholesterol and triglyceride levels (Nakai et al., 2004; Tupin et al., 2004). The increase in atherosclerotic lesion size after α-GalCer administration is attributed to a burst of cytokines (IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-10) in the serum. Initially the NKT cell numbers in the spleen and liver drop, shifting to an increase in the spleen and the aorta after 3 days (Tupin et al., 2004). The initial drop in NKT cells might be caused by an increased recruitment of NKT cells from the spleen and liver to the atherosclerotic lesions where they become activated and secrete IFN-γ and IL-4. Subsequently, a bystander activation of macrophages and endothelial cells in the lesion might occur since an increased VCAM-1 and I-A^{b} expression in the lesions was observed (Tupin et al., 2004). In line with the studies with NKT cell deficient mice, α-GalCer treatment of mice with more advanced atherosclerotic lesions at the aortic root did not affect lesion size but increased the instability of lesions, characterized by a lower collagen content and an increased cellularity (Nakai et al., 2004).

4.2. α-GalCer as anti-atherogenic factor

Immediately after a single injection of α-GalCer, NKT cells secrete large amounts of pro-inflammatory cytokines (Th1 profile) as shown in both naïve mice (Tupin et al., 2004) and mice with established atherosclerotic lesions (Major et al., 2004). Repeated injections of α-GalCer led to undetectable levels of the cytokines in the serum although spleen mRNA expression analysis still showed increased levels of IFN-γ and IL-4 (Tupin et al., 2004). On a longer term (i.e. 2 weeks after the administration), multiple injections of α-GalCer lead to a Th2 cytokine profile of NKT cells in which increased levels of IL-4 and IL-10 were detected with only a slight increase in IFN-γ levels (Major et al., 2004). In line with this cytokine profile, but in contrast with the other studies which suggest a pro-atherogenic effect of α-GalCer treatment, we found that a mixed intravenous (i.v.) and intraperitoneal (i.p.) administration of α-GalCer increased the production of IL-4 and especially IL-10 in the spleen and lymph nodes. These anti-inflammatory cytokines contributed to a reduced thickening of the intima in carotid arteries of LDLr$^{-/-}$ mice, but not apoE$^{-/-}$ mice using a perivascular collar model which, in combination with hypercholesterolemia (9 weeks of high fat diet), induces atherosclerotic lesion development in the carotid arteries

(van Puijvelde et al., 2009). The most striking difference with other studies on α-GalCer and atherosclerosis might be that we examined the effects of α-GalCer in LDLr$^{-/-}$ mice, while other studies only used apoE$^{-/-}$ mice. Both apoE and the LDLr are important mediators of lipid transport and mediate in the presentation of lipids on CD1d and mice deficient in these lipid-transfer related molecules might therefore have a differentially disturbed behaviour of NKT cells leading to differences in the responsiveness to α-GalCer (Elzen et al., 2005). In addition, as mentioned in a study by Braun et al. the iNKT cells in hyperlipidemic mice might be anergic due to a chronic activation by endogenous ligands (Braun et al., 2010). Differences in lipid levels and thus in the anergic state between apoE$^{-/-}$ mice and LDLr$^{-/-}$ mice might also explain the different outcomes between both models upon treatment with α-GalCer.

4.3. OCH

Even more unexpected were the effects of a treatment with (2S,3S,4R)−1-O- (α-D-Galactopyranosyl)-N-tetracosanoyl-2-amino-1,3,4-nonanetriol (OCH), a truncated analogue of α-GalCer, known for the induction of a Th2-cytokine profile by NKT cells (Bricard et al., 2010). While administration of OCH led to an anti-inflammatory cytokine-dependent protection against arthritis (Chiba et al., 2004), colitis (Ueno et al., 2005), insulitis and diabetes (Mizuno et al., 2004), OCH-treatment of apoE$^{-/-}$ mice led to an increase in atherosclerotic lesion size at the aortic root when compared with a vehicle control, but the lesions were smaller than in α-GalCer-treated mice (Nakai et al., 2004). Both ligands induced a similar IL-4 production, but α-GalCer induced more IFN-γ. However, we now showed that treatment of LDLr$^{-/-}$ mice with OCH-pulsed DCs strongly reduced the development of atherosclerotic lesions due to increased levels of IL-10 and a lowering in cholesterol (Puijvelde et al., submitted for publication).

4.4. DPPE-PEG

More recently, and in line with the study on the role of NKT cell derived Granzyme B and perforin in necrotic core formation during atherosclerosis (Li et al., 2015), Li et al. showed that treatment of apoE$^{-/-}$ mice with 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N[methoxy (polyethyleneglycol)−350 (DPPE-PEG$_{350}$) effectively reduced necrotic core size during both the development and progression of hyperlipidaemia-induced atherosclerosis (Li et al., 2016). DPPE-PEG is a lipid antagonist and inhibits the activation of iNKT cells by preventing the presentation of activating ligands on CD1d (Lombardi et al., 2010). Blocking the NKT cell activity by DPPE-PEG$_{350}$ concomitantly reduced the attraction of CD4$^+$ T cells, CD8$^+$ T cells and B cells to the lesion, suggesting an important role of activated NKT cells in the recruitment of other inflammatory cells into the lesion. They now suggest that the reduction in necrosis upon DPPE-PEG$_{350}$ treatment also leads to lower levels of damage-associated molecular patterns such as the pro-atherogenic high mobility group box 1 (HMGB1) (Kanellakis et al., 2011). Conclusively, DPPE-PEG$_{350}$ is an interesting candidate to target iNKT cells in pathologies in which NKT cells worsen the disease such as cardiovascular diseases and atherosclerosis in particular (Li et al., 2016).

5. Lipid-induced NKT cell activation

5.1. α-GalCer and analogues

Since NKT cells are involved in the development of atherosclerotic lesions, the question raised which (modified) endogenous ligand might be responsible for the systemic and local activation of the NKT cells. A lot of research has been done on the glycolipids which are presented on CD1d. These glycolipids mainly consist out of two major parts attached to each other via a glycosidic bond: a lipid part which binds to the hydrophobic binding site within the binding pocket of CD1d and a hydrophilic carbohydrate head group which is exposed for recognition by TCRs. Several studies have now shown that minor modifications to the α-GalCer structure are well tolerated and still give NKT cell activating ligands, however with differences in the cytokine-release profile. While α-GalCer induces a mixed Th1 and Th2 cytokine secretion, a small modification in the glycosidic bond leading to α-C-GalCer, induces especially secretion of Th1 cytokines (IFN-γ) while a truncated analogue of α-GalCer, OCH, as mentioned before preferentially stimulates the secretion of Th2 cytokines (IL-4 and IL-10). These variations in cytokine profile are presumably the consequence of differences in the stability of CD1d-glycolipid complexes. A more stabilized CD1d-glycolipid complex is formed with α-C-GalCer than with OCH and since IFN-γ production requires a more prolonged TCR stimulation than IL-4 and IL-10, binding of α-C-GalCer resulted in the selective production of IFN-γ, while OCH biased the production of IL-4 and IL-10 (Miyamoto et al., 2001; Oki et al., 2004; Yang et al., 2004).

5.2. β-linked glycolipids

Although α-anomeric glycolipids such as α-GalCer are potent agonists of murine and human iNKT cells, it was generally assumed that it is unlikely that they are the endogenous ligands for NKT cells, since these glycolipids are rarely found in humans and other mammalian species (Tsuji, 2006). Extensive research has led to the identification of two β-linked glycolipids, lysosomal isoglobotrihexosylceramide (iGb3) (Zhou et al., 2004b) and disialoganglioside GD3 (Wu et al., 2003) able to activate human and mammalian iNKT cells ligands and both linked to atherosclerosis. GD3 is induced in atherosclerotic tissues and in the circulation of apoE$^{-/-}$ mice (Garner et al., 2002; Mukhin et al., 1995) while intermediates of the synthesis of iGb3, β-GlcCer and lactosylceramide accumulate in the human atherosclerotic lesions (Mukhin et al., 1995). It is still under debate whether these ligands are indeed the endogenous agonist for NKT cells. More recent studies have now shown that, despite the assumption that they cannot produce α-anomeric glycolipids, mammalian immune cells produce constitutively low amounts of α-glycosylceramides, controlled by catabolic enzymes of the ceramide and glycolipid pathways (Kain et al., 2014). So it remains elusive which glycolipid is the endogenous ligand for NKT cells, especially during atherosclerosis.

5.3. Bacteria-derived glycolipids

The majority of the identified iNKT cell ligands are bacteria-derived lipids. Microbial lipids, such as glycosphingolipid-1 (GSL-1) derived from *Sphingomonas* (Long et al., 2007) and *Ehrlichia muris* (Mattner et al., 2005), α-galactosyldiacylglycerol derived from *Borrelia burgdorferi* (Kinjo et al., 2006) and cholesteryl α-glucoside from *Helicobacter pylori* (Chang et al., 2011) have been shown to be recognized by iNKT cells. During bacterial infections, NKT cells play a protective role in the host defence by actively contributing to the clearance of the pathogens by different mechanisms. NKT cells might be activated directly by recognition of the above mentioned glycolipid antigens upon which they secrete cytokines, presumably IFN-γ, which stimulates macrophages to secrete cytokines and actively phagocytose the pathogens, or indirectly via LPS in which endogenous NKT cell ligands and cytokines are involved (De Libero et al., 2005; Mattner et al., 2005). LPS might inhibit an enzyme which normally degrades iGb3, α-galactosidase A, thereby elevating iGb3 levels, and TLR signalling leads to increased levels of the endogenous ligand β-GlcCer, both leading to enhanced iNKT cell activation (Brennan et al., 2011; Darmoise et al., 2010).

5.4. Phospholipids

Phospholipids are another important class of lipids with reported

NKT cell stimulating capacity. The polar phosphate group of these NKT cell activating ligands are generally linked to either an inositol group, phosphatidylinositol (PI) such as phosphatidylinositol tetramannosides (PIM4) from *Mycobacterium bovis* (Zajonc et al., 2006), or to a choline (-like) group as seen in phosphatidylcholine (PC) (Giabbai et al., 2005) and phosphatidylethanolamine (PE) (Joyce et al., 1998). Phospholipids and oxidized derivatives are essential constituents of LDL and oxLDL respectively. Upon exposure to oxLDL and lysophosphatidic acid, macrophages and DCs increase the expression of CD1d in a peroxisome proliferator activated receptor (PPAR)γ-mediated way (Leslie et al., 2008; Szatmari et al., 2006). Together with an upregulation of TLR4 this subsequently enhances the activation of NKT cells resulting in increased production of IFN-γ. These findings are important in the context of atherosclerosis since lipids are abundantly present in the lesions and NKT cells exacerbate atherosclerosis in LPS-treated mice (Andoh et al., 2013; Nakai et al., 2004). VanderLaan et al. showed that LDL present in serum of LDLr$^{-/-}$ mice on a high fat diet contains an "endogenous" antigen able to stimulate Vα14Jα18-expressing hybridoma cells (NKT hybridoma). Loading of the antigens onto CD1d is dependent on endocytosis facilitated by the LDL receptor. However, upon extensive oxidation of LDL the endocytosis via the LDL receptor is impaired, impairing the NKT cell stimulating capacity of LDL (VanderLaan et al., 2007). The precise nature of the NKT cell activating endogenous lipid in LDL remained however elusive since LDL is a complex particle able to transport a wide range of lipids including phospholipids. Fox et al. now showed that lysophosphatidylcholine, a major phospholipid component of oxLDL with pro-atherogenic activity, is recognized by human NKT cells (Fox et al., 2009), but presumably not by mouse NKT cells (Brennan et al., 2011). Structure comparison analysis with existing NKT cell ligands led to the presumption that in addition to lysophosphatidylcholine, palmitoyl oleoyl phosphatidylcholine (POPC), palmitoyl linoleoyl phosphatidylcholine (PLPC), palmitoyl arachidonoyl phosphatidylcholine (PAPC), saturated dipalmitoylphosphatidylcholine (DPPC) and palmitoyl docosahexaenoyl phosphatidylcholine (PDPC) could be able to stimulate iNKT cells. Unpublished data from our group now shows that oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoryl-choline (oxPAPC), present in oxLDL, has the capacity to stimulate NKT cells (Puijvelde et al., submitted for publication).

6. Relation between lipoprotein metabolism and NKT cells

Loading of (endogenous) (glyco)lipid-antigens, which are mostly present in cell-membranes, on CD1d is a complex process still under investigation. CD1d is synthesized in the endoplasmic reticulum (ER) and loading of lipid ligands occurs in the endosomes and lysosomes. Several proteins that are involved in lipoprotein metabolism and linked to atherosclerosis are also involved in the homeostasis and lipid-driven activation of NKT cells. Microsomal triglyceride transfer protein (MTP), a lipid transfer protein involved in the assembly of VLDL and chylomicrons by loading lipids onto apolipoprotein B, also regulates loading of glycolipid antigens onto CD1d and the subsequent presentation of the loaded-CD1d molecules on the surface of APCs (Brozovic et al., 2004; Dougan et al., 2005). MTP inhibitors attenuate atherosclerosis (Hewing et al., 2013; Mera et al., 2015) due to a reversal of hyperlipidaemia but the reduction in lesion size might also be caused by a reduced loading of lipids on CD1d and subsequently in inhibited activation of pro-atherogenic NKT cells. Elevated levels of GM2 activator protein, a sphingolipid activator protein (SAP), are found in serum and atherosclerotic lesions of mice and humans (Yanai et al., 2006). This lysosomal protein activates lipid-degrading enzymes and increases the enzymes' accessibility to lipids in the lysosomes and endosomes. Consequently, SAPs can promote the loading of lipids on CD1d which could explain (Zhou et al., 2004a), at least partly, how these proteins contribute to atherosclerosis.

6.1. ApoE-LDLr pathway

A very important lipid transfer pathway involving lipoproteins and linked to atherosclerosis in both mice and humans is the ApoE-LDLr pathway. ApoE is involved in the clearance of lipids, especially chylomicrons, VLDL and IDL particles from the circulation via the interaction with the LDLr or LDLr-related protein (LRP). LRP, highly expressed on macrophages and involved in intracellular signalling and endocytosis, can modulate the activation of NKT cells, but is only necessary for the IL-4 and not the IFN-γ production (Covarrubias et al., 2014). Van den Elzen et al. revealed an important role for both apoE and the LDLr in the CD1d-mediated presentation of exogenous NKT cell antigens. ApoE is necessary for the effective delivery of these antigens to the lysosomes where they can be loaded onto CD1d and lead to iNKT cell activation. This process is receptor-mediated in which the LDLr is an important factor (Elzen et al., 2005). Although both apoE and the LDLr are not essential for NKT cell activation, a deficiency in one of them leads to a dramatic decrease in NKT cell activation. NKT cell activation by B cells is however completely LDLr-dependent and apoE dramatically enhances B cell presentation of α-GalCer (Allan et al., 2009). As a consequence there are major differences in the α-GalCer-sensitivity under apoE$^{-/-}$ and LDLr$^{-/-}$ conditions. NKT cells in apoE$^{-/-}$ conditions proliferate less upon stimulation with α-GalCer (both in vitro and in vivo) suggesting that apoE might be more important than the LDLr in lipid-loading of CD1d. In addition, the increase in splenic and hepatic NKT cell numbers upon high fat diet feeding was much lower in apoE$^{-/-}$ mice than in LDLr$^{-/-}$ mice. ApoE might therefor not only be important in transport of exogenously administered ligands but also in the transport of atherosclerosis-related endogenous NKT cell ligands (van Puijvelde et al., 2009). In accordance, Braun et al. showed an impaired response to α-GalCer in apoE$^{-/-}$ splenocytes compared with WT splenocytes. As mentioned, another explanation for this can be the anergic state of iNKT cells during hyperlipidaemia caused by a chronic activation by endogenous ligands (Braun et al., 2010). Based upon this knowledge we should however conclude that the mice-models (apoE$^{-/-}$ and LDLr$^{-/-}$ mice) used in all studies on atherosclerosis are not ideal to examine the contribution of NKT cells. To circumvent the problems with CD1d-loading due to the deficiency in apoE or LDLr, exogenous ligands could be loaded on wildtype (C57Bl/6) DCs followed by intravenous injection, a method shown to be even more effective in the activation of NKT cells than direct injection of α-GalCer (Chang et al., 2005; Nagaraj et al., 2006). As mentioned, treatment of LDLr$^{-/-}$ mice with OCH-pulsed DCs strongly reduced the development of atherosclerotic lesions (Puijvelde et al., submitted for publication). Another effective approach could be the incorporation of lipid-ligands in liposomes (Nakamura et al., 2015), shown to induce strong NKT cell responses and improved antitumor effects (Nakamura et al., 2013). However, in studies with hyperlipidemic mice, you still have to deal with the possible anergic state of the NKT cells. Soh et al. very recently showed that this anergic state of NKT cells with reduced capacity to produce IFN-γ, leads to an accumulation of pro-atherogenic marginal zone B cells (Soh et al., 2016).

7. NKT cells in other cardiovascular diseases and patients

Since NKT cells are involved in the disease process of atherosclerosis, the question was raised whether the prevalence or behaviour of circulating NKT cells is also altered in patients with cardiovascular diseases. In Table 1 and below, we briefly discuss the current knowledge on the prevalence and the role of NKT cells in several cardiovascular diseases and events.

7.1. Coronary artery disease

Similar to the reduction of NKT cell numbers in spleen and liver of

Table 1
Prevalence of NKT cells in cardiovascular diseases.

Disease	Species	NKT cell type	Circulation	Local	Pathologic function	Refs.
Atherosclerosis	Mice	Type I NKT cells ($TCR\alpha\beta^+NK1.1^+$ NKT cells)	Unchanged	Decreased in liver and spleen, increased in atherosclerotic plaques	NKT cells produce relatively more cytokines	Major et al. (2004), Nakai et al. (2004)
	Mice	$CD3^+NK1.1^+$ NKT cells		Increased in liver and spleen		Puijvelde et al. (2009)
	Mice	Type I NKT cells		Increased in aortic arches		Aslanian et al. (2005)
	Human	$CD3^+CD7^+$ NKT cells	Lowered	Increased in atherosclerotic plaques	Increased inflammation in the plaque	Rombouts et al. (2016)
Angina pectoris	Human	Type I NKT cells	Lowered			Andoh et al. (2006)
Symptomatic cardiovascular disease	Human	Type I NKT cells	Lowered	Increased in atherosclerotic plaques	Highly reactive to α-GalCer and promote neovascularization	Kyriakakis et al. (2010)
Coronary Artery Disease	Human	$CD8^+CD56^+$ NKT like	Accumulate		Increased IFN-γ concentration in blood	Bergström et al. (2012)
	Human	$CD3^+CD4+CD56^+$ NKT like	Lowered			Björkbacka et al. (2016)
Ischemia/Reperfusion Injury	Mice	$CD8^+$ NKT cells		Increased in kidney	Increased tissue injury	Tsutahara et al. (2012)
	Mice	CD1d restricted NKT cells		Increased in liver	Increased tissue injury	Caldwell et al. (2005), Shimamura et al. (2005)
Stroke	Mice	Type I NKT cells	Unchanged	Increased arrest in liver	Reduced protection against post-stroke infections	Wong et al. (2011)
Myocardial Infarction	Mice	$CD3^+NK1.1^+$ NKT cells		Increased in affected heart		Yan et al. (2013)
	Human	Type I NKT cells	Lowered	Increased in atherosclerotic plaques		Liu et al. (2011); Novak et al. (2015)
Abdominal Aortic Aneurysm	Human	CD4+TCRαβ+CD161+ NKT cells		Increased in AAA tissue	Increased IFN-γ and IL-4 production, MMP-expression and VSMC apoptosis	Chan et al. (2005a)

mice upon the induction of atherosclerosis (Major et al., 2004; Nakai et al., 2004), and similar to the reduced numbers observed in many autoimmune diseases (Kis et al., 2007; van der Vliet et al., 2001; Yanagihara et al., 1999), patients with angina pectoris (Andoh et al., 2006) and symptomatic cardiovascular patients, patients who experienced cardiovascular events in the past (Kyriakakis et al., 2010), show lower circulating levels of NKT cells. Advanced vascularized atherosclerotic plaques from the symptomatic patients showed increased numbers of CD1d-expressing cells when compared with early plaque stages or with plaques of asymptomatic patients. This again suggests an active migration of NKT cells from the periphery to atherosclerotic lesions. Furthermore, the iNKT cells from these plaques co-localize with CD1d-expressing cells, are highly reactive to α-GalCer and promote angiogenesis and further neovascularization by producing IL-8 (Kyriakakis et al., 2010). Although a correlation between circulating NKT cell numbers and coronary risk factors could not be established, the prevalence of NKT cells might be a good biomarker for atherosclerotic coronary artery disease (Andoh et al., 2006). A recent paper by Rombouts et al. now showed a very strong correlation between circulating NKT cell levels and intraplaque T and NKT cells, confirming the potential of circulating NKT cells as a biomarker for inflammatory atherosclerotic lesions (Rombouts et al., 2016). However, more extended studies with bigger groups of patients should be performed to confirm this. On the contrary, $CD8^+CD56^+$ NKT and NKT-like cells producing pro-atherogenic IFN-γ persistently accumulate in blood from patients with coronary artery disease (acute coronary syndrome and stable angina) (Bergström et al., 2012) and low levels of IFN-γ expressing $CD3^+CD4^+CD56^+$ NKT and NKT-like cells were linked to an increased incidence of coronary events which may be lipoprotein-dependent (Björkbacka et al., 2016).

7.2. Ischemia/Reperfusion injury

NKT cells are also associated with tissue injury after ischemia reperfusion (I/R), a disease process linked to atherosclerosis. IFN-γ-producing CD1d-restricted NKT cells are rapidly recruited to the kidney and liver after ischemia/reperfusion induction in mice models (Caldwell et al., 2005; Shimamura et al., 2005; Tsutahara et al., 2012). The recruitment of $CD8^+$ NKT cells to the injured kidney is a CXCR3 and CCR5 dependent process (Tsutahara et al., 2012). The injury is initiated by CD1d-dependent activation of the NKT cells and can be inhibited by the activation of the hypoxia/HIF-2α/adenosine A_{2A} receptor axis of iNKT cells, leading to a reduced IFN-γ production, neutrophil influx and tissue necrosis (Lappas et al., 2006; Zhang et al., 2016). The NKT cells also contribute directly to the liver and renal injury by their cytotoxic, perforin and Fas/FasL-dependent function (Shimamura et al., 2005).

Mice in which NKT cells were depleted using an anti-CD1d mAb, blocking the interaction between APCs and NKT cells, and mice deficient in type I NKT cells ($J\alpha18^{-/-}$ mice) showed reduced hepatic and renal I/R injury (Arrenberg et al., 2011; Kuboki et al., 2009; Li et al., 2007). A reduced IFN-γ production and a reduced neutrophil accumulation were responsible for the protection against renal injury in these mice (Li et al., 2007). Additionally, α-GalCer mediated activation of NKT cells intensifies renal I/R injury in which both the IL-12/IFN-γ and the IL-23/IL-17 signal pathways are involved (Li et al., 2010). However, interestingly and surprisingly, treatment of mice with α-GalCer resulted in an IL-13 and adenosine A_{2A} receptor-dependent reduction in hepatic I/R injury (Cao et al., 2009).

In contrast with the pathogenic role of type I NKT cells in I/R injury, sulfatide-reactive type II NKT cells are now linked to a IL-10 and HIF-1α dependent protective role in liver and kidney injury after I/R (Arrenberg et al., 2011; Yang et al., 2011). In addition, Zhang et al. showed that treatment with rapamycin, an immunosuppressing drug used in preventing the rejection of kidney transplants, protects the kidney against I/R injury in an early stage through an active chemo-

kine-dependent recruitment of renoprotective NKT cells. Further research should however prove whether these NKT cells are indeed the same type II NKT cells (Zhang et al., 2014).

Wong et al. showed that NKT cells also play a role in ischemic reperfusion injury of the brain (stroke) and especially in the associated systemic bacterial infection. Stroke induces a restricted crawling and increased arrest of hepatic iNKT cells in mice which might cause the attenuated antimicrobial responses as observed in patients. Surprisingly, these effects were not dependent on a CD1d ligand but were caused by a noradrenergic neurotransmitter (Wong et al., 2011).

7.3. Myocardial infarction

In line with the other studies (Andoh et al., 2006; Kyriakakis et al., 2010) patients with acute myocardial infarction (MI) show reduced numbers of circulating NKT cells when compared with healthy controls. This again might be a result of an increased migration of peripheral NKT cells to the site of inflammation, the infarcted heart (Liu et al., 2011; Novak et al., 2015), where they peak seven days after MI induction (Yan et al., 2013). During MI there is however no change in the proportion of subpopulations of iNKT cells, but there is a change in the expression of NK cell receptors leading to more pathogenic NKT cells promoting inflammation. CD244, linked to both the activation and the inhibition of NK cells, is downregulated while CD16, playing a role in NK cell activation, is upregulated (Novak et al., 2015). A correlation analysis by Liu et al. now showed that the number of iNKT cells might be a predictor of restenosis after primary coronary stenting in acute MI patients (Liu et al., 2011). Homma et al. and Sobirin et al. showed that α-GalCer-induced activation of NKT cells in mice results in a concomitant increase in cardiac NKT cell numbers. Accompanied by a reduction in the inflammatory status and apoptosis this leads to a reduction in myocardial I/R injury and an amelioration of left ventricle remodelling which were both IL-10 dependent and concomitantly resulted in an improved survival (Homma et al., 2013; Sobirin et al., 2012). Further investigation on the applicability of a α-GalCer based therapy to protect the heart from I/R injury is needed since enhanced NKT cell infiltration in the ischemic heart and particularly in the left ventricle after I/R (Homma et al., 2013; Sobirin et al., 2012; Yan et al., 2013) and a reduction in infarct size in iNKT cell deficient mice are observed (Homma et al., 2013).

7.4. Aortic aneurysms

NKT cells are also found in vascular tissue affected by another common vascular disorder, the abdominal aortic aneurysm (AAA). AAA, a local permanent dilation of the abdominal part of the aorta, is linked to aging and atherosclerosis. AAA is a chronic inflammatory disease in which inflammatory cells contribute to an increased production of elastase and several proteinases (matrix metalloproteinases (MMPs), serine proteinases, cathepsins) which are responsible for the structural loss of vessel wall integrity. $TCR\alpha B^{+}CD161^{+}$ NKT cells are one of the inflammatory cells that can be found abundantly in the aneurysmal vessel wall. NKT cells present in AAA tissue may be activated via a CD40/CD40L interaction with vSMCs. Upon activation, the NKT cells predominantly produce pro-inflammatory IFN-γ which may lead to an upregulated expression of Fas and increased FasL-mediated apoptosis of vascular smooth muscle cells (vSMCs) leading to atherosclerotic plaque instability and rupture (Chan et al., 2005a). Anti-inflammatory IL-4, produced by the same NKT cells, might be responsible for increased expression of MMPs by SMCs and macrophages, contributing to the development of AAA (Chan et al., 2005b). Data from our group now establishes that mice lacking CD1d-dependent NKT cells demonstrate reduced AAA severity in the angiotensin-II-mediated AAA model in $LDLr^{-/-}$ mice. In addition, we show that type I NKT cells can contribute, in a cytokine dependent way, to AAA development by increasing the expression of matrix degrading enzymes by macrophages and vSMCs, and by decreasing vSMC viability (Puijvelde et al., submitted for publication).

8. Concluding remarks

Although the $LDLr^{-/-}$ and $apoE^{-/-}$ mouse models used are far from ideal, it is generally accepted that iNKT cells are pro-atherogenic and may affect the initiation (size) as well as the progression of atherosclerotic lesion development (stability). Initial studies in mice on the role of NKT cells in atherosclerosis mainly focused on the cytokines (IFN-γ, IL-4 and IL-10) produced upon activation, while more recent papers now emphasize the importance of the cytotoxic capabilities of NKT cells. Granzyme B and perforin, released by NKT cells may contribute to the necrotic core formation, resulting in rupture-prone instable lesions. The role of NKT cells in humans with cardiovascular diseases remains however less clear. Overall, most cardiovascular patients show a reduction in circulating NKT cells and a concomitant increase in NKT cells in the affected organ. From a study by Rombouts et al. we now learned that circulating NKT cells strongly correlate with (NK)T cells inside the lesion, and thus might be a biomarker for the presence of highly inflammatory lesions in both mice and humans (Rombouts et al., 2016). The recruited NKT cells are often linked to a progression of the disease (I/R injury, aneurysm formation, atherosclerosis, coronary artery disease) but might also be protective, especially when activated by α-GalCer. Further investigation is necessary to define the best strategy, most likely a dampening (DPPE-PEG, anti-CD1d) or a modulation (structural variants of α-GalCer with beneficial anti-inflammatory effects) of the NKT cell responses, as a potential novel therapy against cardiovascular diseases. Extra complicating is however that the NKT cell activity is highly appreciated in cancer because of the anti-tumor activity. Therefore, the discovery of the so far unknown endogenous ligand for NKT cells during cardiovascular diseases might have great impact on possible therapies. Extensive research on these endogenous ligand (s) led to some interesting candidates, but whether these ligands (iGb3, GD3, α-glycosylceramides) indeed activate NKT cells during cardiovascular diseases remains elusive.

Funding

The authors received support from the European Union's Seventh Framework Programme (FP7/2007–2013) under grant agreement VIA no. 603131.

Acknowledgements

We would like to thank Servier for the free of charge use of their Medical Art images which can be found on http://servier.com/Powerpoint-image-bank.

References

Afek, A., George, J., Gilburd, B., Rauova, L., Goldberg, I., Kopolovic, J., Harats, D., Shoenfeld, Y., 2000. Immunization of low-density lipoprotein receptor deficient (LDL-RD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis. J. Autoimmun. 14, 115–121. http://dx.doi.org/10.1006/jaut.1999.0351.

Agrati, C., Martini, F., Nisii, C., Oliva, A., D'Offizi, G., Narciso, P., Nardacci, R., Piacentini, M., Dieli, F., Pucillo, L.P., Poccia, F., 2005. CD1d expression by hepatocytes is a main restriction element for intrahepatic T-cell recognition. J. Biol. Regul. Homeost. Agents 19, 41–48.

Allan, L.L., Hoefl, K., Zheng, D.-J., Chung, B.K., Kozak, F.K., Tan, R., van den Elzen, P., 2009. Apolipoprotein-mediated lipid antigen presentation in B cells provides a pathway for innate help by NKT cells. Blood 114, 2411–2416.

Andoh, Y., Fujii, S., Iwabuchi, K., Yokota, T., Inoue, N., Nakai, Y., Mishima, T., Yamashita, T., Nakagawa, T., Kitabatake, A., Onoe, K., Tsutsui, H., 2006. Lower prevalence of circulating natural killer T cells in patients with angina: a potential novel marker for coronary artery disease. Coron. Artery Dis. 17, 523–528.

Andoh, Y., Ogura, H., Satoh, M., Shimano, K., Okuno, H., Fujii, S., Ishimori, N., Eshima, K., Tamauchi, H., Otani, T., Nakai, Y., Kaer, L., Van, Tsutsui, H., Onoé, K., Iwabuchi, K., 2013. Natural killer T cells are required for lipopolysaccharide-mediated enhancement of atherosclerosis in apolipoprotein E-deficient mice. Immunobiology

218, 561–569. http://dx.doi.org/10.1016/j.imbio.2012.07.022.
Arrenberg, P., Halder, R., Dai, Y., Maricic, I., Kumar, V., 2010. Oligoclonality and innate-like features in the TCR repertoire of type II NKT cells reactive to a beta-linked self-glycolipid. Proc. Natl. Acad. Sci. USA 107, 10984–10989. http://dx.doi.org/10.1073/pnas.1000576107.
Arrenberg, P., Maricic, I., Kumar, V., 2011. Sulfatide-mediated activation of type II natural killer T cells prevents hepatic ischemic reperfusion injury in mice. Gastroenterology 140, 646–655. http://dx.doi.org/10.1053/j.gastro.2010.10.003.
Aslanian, A.M., Chapman, H.A., Charo, I.F., 2005. Transient role for CD1d-restricted natural killer T cells in the formation of atherosclerotic lesions. Arterioscler. Thromb. Vasc. Biol. 25, 628–632. http://dx.doi.org/10.1161/01.ATV.0000153046.59370.13.
Benagiano, M., D'Elios, M.M., Amedei, A., Azzurri, A., van der Zee, R., Ciervo, A., Rombolà, G., Romagnani, S., Cassone, A., Del Prete, G., 2005. Human 60-kDa heat shock protein is a target autoantigen of T cells derived from atherosclerotic plaques. J. Immunol. 174, 6509–6517. http://dx.doi.org/10.4049/JIMMUNOL.174.10.6509.
Bergström, I., Backteman, K., Lundberg, A., Ernerudh, J., Jonasson, L., 2012. Persistent accumulation of interferon-γ-producing CD8+CD56+ T cells in blood from patients with coronary artery disease. Atherosclerosis 224, 515–520. http://dx.doi.org/10.1016/j.atherosclerosis.2012.07.033.
Björkbacka, H., Berg, K.E., Manjer, J., Engelbertsen, D., Wigren, M., Ljungcrantz, I., Andersson, L., Hedblad, B., Fredrikson, G.N., Nilsson, J., 2016. CD4 $^{+}$ CD56 $^{+}$ natural killer T-like cells secreting interferon-γ are associated with incident coronary events. J. Intern. Med. 279, 78–88. http://dx.doi.org/10.1111/joim.12392.
Bobryshev, Y.V., Lord, R.S.A., 2002. Expression of heat shock protein–70 by dendritic cells in the arterial intima and its potential significance in atherogenesis. J. Vasc. Surg. 35, 368–375. http://dx.doi.org/10.1067/mva.2002.121067.
Bobryshev, Y.V., Lord, R.S.A., 2005. Co-accumulation of dendritic cells and natural killer T cells within rupture-prone regions in human atherosclerotic plaques. J. Histochem. Cytochem. 53, 781–785. http://dx.doi.org/10.1369/jhc.4B6570.2005.
Bourgeois, E., Van, L.P., Samson, M., Diem, S., Barra, A., Roga, S., Gombert, J.-M., Schneider, E., Dy, M., Gourdy, P., Girard, J.-P., Herbelin, A., 2009. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-γ production. Eur. J. Immunol. 39, 1046–1055. http://dx.doi.org/10.1002/eji.200838575.
Braun, N.A., Mendez-Fernandez, Y.V., Covarrubias, R., Porcelli, S.A., Savage, P.B., Yagita, H., Van Kaer, L., Major, A.S., 2010. Development of spontaneous anergy in invariant natural killer T cells in a mouse model of dyslipidemia. Arterioscler. Thromb. Vasc. Biol. 30, 1758–1765. http://dx.doi.org/10.1161/ATVBAHA.110.206045.
Brennan, P.J., Tatituri, R.V.V., Brigl, M., Kim, E.Y., Tuli, A., Sanderson, J.P., Gadola, S.D., Hsu, F.-F., Besra, G.S., Brenner, M.B., 2011. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. Nat. Immunol. 12, 1202–1211. http://dx.doi.org/10.1038/ni.2143.
Bricard, G., Venkataswamy, M.M., A Yu, K.O., Im, J.S., Ndonye, R.M., Howell, A.R., Veerapen, N., Illarionov, P.A., Besra, G.S., Li, Q., Chang, Y.-T., Porcelli, S.A., 2010. α-Galactosylceramide analogs with weak agonist activity for human iNKT cells define new candidate anti-inflammatory agents. PLoS One 5, e14374. http://dx.doi.org/10.1371/journal.pone.0014374.
Brozovic, S., Nagaishi, T., Yoshida, M., Betz, S., Salas, A., Chen, D., Kaser, A., Glickman, J., Kuo, T., Little, A., Morrison, J., Corazza, N., Kim, J.Y., Colgan, S.P., Young, S.G., Exley, M., Blumberg, R.S., 2004. CD1d function is regulated by microsomal triglyceride transfer protein. Nat. Med. 10, 535–539. http://dx.doi.org/10.1038/nm1043.
Caldwell, C.C., Okaya, T., Martignoni, A., Husted, T., Schuster, R., Lentsch, A.B., 2005. Divergent functions of CD4+ T lymphocytes in acute liver inflammation and injury after ischemia-reperfusion. Am. J. Physiol. Gastrointest. Liver Physiol. 289, G969–G976.
Canchis, P.W., Bhan, A.K., Landau, S.B., Yang, L., Balkt, S.P., Blumberg, R.S., 1993. Tissue distribution of the non-polymorphic major histocompatibility complex class I-like molecule, CD1d. Immunology 80, 561–565.
Cao, Z., Yuan, Y., Jeyabalan, G., Du, Q., Tsung, A., Geller, D.A., Billiar, T.R., 2009. Preactivation of NKT cells with alpha-GalCer protects against hepatic ischemia-reperfusion injury in mouse by a mechanism involving IL-13 and adenosine A2A receptor. Am. J. Physiol. Gastrointest. Liver Physiol. 297, G249–G258. http://dx.doi.org/10.1152/ajpgi.00041.2009.
Carnaud, C., Lee, D., Donnars, O., Park, S.-H., Beavis, A., Koezuka, Y., Bendelac, A., 1999. Cutting edge: cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J. Immunol. 163, 4647–4650.
Chan, W.L., Pejnovic, N., Hamilton, H., Liew, T.V., Popadic, D., Poggi, A., Khan, S.M., 2005a. Atherosclerotic abdominal aortic aneurysm and the interaction between autologous human plaque-derived vascular smooth muscle cells, type 1 NKT, and helper T cells. Circ. Res. 96, 675–683. http://dx.doi.org/10.1161/01.RES.0000160543.84254.f1.
Chan, W.L., Pejnovic, N., Liew, T.V., Hamilton, H., 2005b. Predominance of Th2 response in human abdominal aortic aneurysm: mistaken identity for IL-4-producing NK and NKT cells? Cell. Immunol. 233, 109–114. http://dx.doi.org/10.1016/j.cellimm.2005.04.020.
Chan, W.L., Pejnovic, N., Liew, T.V., Lee, C.A., Groves, R., Hamilton, H., 2003. NKT cell subsets in infection and inflammation. Immunol. Lett. 85, 159–163.
Chang, D.H., Osman, K., Connolly, J., Kukreja, A., Krasovsky, J., Pack, M., Hutchinson, A., Geller, M., Liu, N., Annable, R., Shay, J., Kirchhoff, K., Nishi, N., Ando, Y., Hayashi, K., Hassoun, H., Steinman, R.M., Dhodapkar, M.V., 2005. Sustained expansion of NKT cells and antigen-specific T cells after injection of alpha-galactosyl-ceramide loaded mature dendritic cells in cancer patients. J. Exp. Med. 201, 1503–1517. http://dx.doi.org/10.1084/jem.20042592.
Chang, Y.-J., Kim, H.Y., Albacker, L.A., Lee, H.H., Baumgarth, N., Akira, S., Savage, P.B., Endo, S., Yamamura, T., Maaskant, J., Kitano, N., Singh, A., Bhatt, A., Besra, G.S., van den Elzen, P., Appelmelk, B., Franck, R.W., Chen, G., DeKruyff, R.H., Shimamura, M., Illarionov, P., Umetsu, D.T., 2011. Influenza infection in suckling mice expands an NKT cell subset that protects against airway hyperreactivity. J. Clin. Investig. 121, 57–69. http://dx.doi.org/10.1172/JCI44845.
Chiba, A., Oki, S., Miyamoto, K., Hashimoto, H., Yamamura, T., Miyake, S., 2004. Suppression of collagen-induced arthritis by natural killer T cell activation with OCH, a sphingosine-truncated analog of α-galactosylceramide. Arthritis Rheum. 50, 305–313. http://dx.doi.org/10.1002/art.11489.
Choy, J.C., McDonald, P.C., Suarez, A.C., Hung, V.H.Y., Wilson, J.E., McManus, B.M., Granville, D.J., 2003. Granzyme B in atherosclerosis and transplant vascular disease: association with cell death and atherosclerotic disease severity. Mod. Pathol. 16, 460–470. http://dx.doi.org/10.1097/01.MP.0000067424.12280.BC.
Coppieters, K., Van Beneden, K., Jacques, P., Dewint, P., Vervloet, A., Vander Cruyssen, B., Van Calenbergh, S., Chen, G., Franck, R.W., Verbruggen, G., Deforce, D., Matthys, P., Tsuji, M., Rottiers, P., Elewaut, D., 2007. A single early activation of invariant NK T cells confers long-term protection against collagen-induced arthritis in a ligand-specific manner. J. Immunol. 179, 2300–2309. http://dx.doi.org/10.4049/JIMMUNOL.179.4.2300.
Covarrubias, R., Wilhelm, A.J., Major, A.S., 2014. Specific deletion of LDL receptor-related protein on macrophages has skewed in vivo effects on cytokine production by invariant natural killer T cells. PLoS One 9, e102236. http://dx.doi.org/10.1371/journal.pone.0102236.
Darmoise, A., Teneberg, S., Bouzonville, L., Brady, R.O., Beck, M., Kaufmann, S.H.E., Winau, F., 2010. Lysosomal alpha-galactosidase controls the generation of self lipid antigens for natural killer T cells. Immunity 33, 216–228. http://dx.doi.org/10.1016/j.immuni.2010.08.003.
De Libero, G., Moran, A.P., Gober, H.-J., Rossy, E., Shamshiev, A., Chelnokova, O., Mazorra, Z., Vendetti, S., Sacchi, A., Prendergast, M.M., Sansano, S., Tonevitsky, A., Landmann, R., Mori, L., 2005. Bacterial infections promote T cell recognition of self-glycolipids. Immunity 22, 763–772. http://dx.doi.org/10.1016/j.immuni.2005.04.013.
Dougan, S.K., Salas, A., Rava, P., Agyemang, A., Kaser, A., Morrison, J., Khurana, A., Kronenberg, M., Johnson, C., Exley, M., Hussain, M.M., Blumberg, R.S., 2005. Microsomal triglyceride transfer protein lipidation and control of CD1d on antigen-presenting cells. J. Exp. Med. 202, 529–539. http://dx.doi.org/10.1084/jem.20050183.
Eberl, G., Brawand, P., Macdonald, H.R., Robson Macdonald, H., 2000. Selective bystander proliferation of memory CD4+ and CD8+ T cells upon NK T or T cell activation. J. Immunol. 165, 4305–4311. http://dx.doi.org/10.4049/jimmunol.165.8.4305.
Eberl, G., MacDonald, H.R., 1998. Rapid death and regeneration of NKT cells in anti-CD3ε- or IL-12-treated mice: a major role for bone marrow in NKT cell homeostasis. Immunity 9, 345–353. http://dx.doi.org/10.1016/S1074-7613(00)80617-2.
van den, Elzen, P., Garg, S., León, L., Brigl, M., Leadbetter, E.A., Gumperz, J.E., Dascher, C.C., Cheng, T.-Y., Sacks, F.M., Illarionov, P.A., Besra, G.S., Kent, S.C., Moody, D.B., Brenner, M.B., 2005. Apolipoprotein-mediated pathways of lipid antigen presentation. Nature 437, 906–910. http://dx.doi.org/10.1038/nature04001.
Fox, L.M., Cox, D.G., Lockridge, J.L., Wang, X., Chen, X., Scharf, L., Trott, D.L., Ndonye, R.M., Veerapen, N., Besra, G.S., Howell, A.R., Cook, M.E., Adams, E.J., Hildebrand, W.H., Gumperz, J.E., 2009. Recognition of lyso-phospholipids by human natural killer T lymphocytes. PLoS Biol. 7, e1000228. http://dx.doi.org/10.1371/journal.pbio.1000228.
Fujii, S.-I., Shimizu, K., Smith, C., Bonifaz, L., Steinman, R.M., 2003. Activation of natural killer T cells by α-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. J. Exp. Med 198, 267–279. http://dx.doi.org/10.1084/jem.20030324.
Gansuvd, B., Hagihara, M., Yu, Y., Inoue, H., Ueda, Y., Tsuchiya, T., Masui, A., Ando, K., Nakamura, Y., Munkhtuvshin, N., Kato, S., Thomas, J.M., Hotta, T., 2002. Human umbilical cord blood NK T cells kill tumors by multiple cytotoxic mechanisms. Hum. Immunol. 63, 164–175.
Gapin, L., Matsuda, J.L., Surh, C.D., Kronenberg, M., 2001. NKT cells derive from double-positive thymocytes that are positively selected by CD1d. Nat. Immunol. 2, 971–978. http://dx.doi.org/10.1038/ni710.
Garner, B., Priestman, D.A., Stocker, R., Harvey, D.J., Butters, T.D., Platt, F.M., 2002. Increased glycosphingolipid levels in serum and aortae of apolipoprotein E gene knockout mice. J. Lipid Res. 43, 205–214.
Giabbai, B., Sidobre, S., Crispin, M.D.M., Sanchez-Ruìz, Y., Bachi, A., Kronenberg, M., Wilson, I.A., Degano, M., 2005. Crystal structure of mouse CD1d bound to the self ligand phosphatidylcholine: a molecular basis for NKT cell activation. J. Immunol. 175, 977–984. http://dx.doi.org/10.4049/JIMMUNOL.175.2.977.
Han, J., Hajjar, D.P., Febbraio, M., Nicholson, A.C., 1997. Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36. J. Biol. Chem. 272, 21654–21659. http://dx.doi.org/10.1074/jbc.272.34.21654.
Hansson, G.K., 2001. Immune mechanisms in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 21, 1876–1890.
Hansson, G.K., Holm, J., Jonasson, L., 1989. Detection of activated T lymphocytes in the human atherosclerotic plaque. Am. J. Pathol. 135, 169–175.
Hegde, S., Jankowska-Gan, E., Roenneburg, D.A., Torrealba, J., Burlingham, W.J., Gumperz, J.E., 2009. Human NKT cells promote monocyte differentiation into suppressive myeloid antigen-presenting cells. J. Leukoc. Biol. 86, 757–768. http://dx.doi.org/10.1189/jlb.0209059.
Hewing, B., Parathath, S., Mai, C.K., Fiel, M.I., Guo, L., Fisher, E.A., 2013. Rapid regression of atherosclerosis with MTP inhibitor treatment. Atherosclerosis 227, 125–129. http://dx.doi.org/10.1016/j.atherosclerosis.2012.12.026.
Hiebert, P.R., Boivin, W.A., Zhao, H., McManus, B.M., Granville, D.J., 2013. Perforin and granzyme B have separate and distinct roles during atherosclerotic plaque development in apolipoprotein E knockout mice. PLoS One 8, e78939. http://dx.doi.org/10.1371/journal.pone.0078939.
Homma, T., Kinugawa, S., Takahashi, M., Sobirin, M.A., Saito, A., Fukushima, A., Suga, T., Takada, S., Kadoguchi, T., Masaki, Y., Furihata, T., Taniguchi, M., Nakayama, T., Ishimori, N., Iwabuchi, K., Tsutsui, H., 2013. Activation of invariant natural killer T cells by α-galactosylceramide ameliorates myocardial ischemia/reperfusion injury in mice. J. Mol. Cell. Cardiol. 62, 179–188. http://dx.doi.org/10.1016/

j.yjmcc.2013.06.004.
Hong, S., Wilson, M.T., Serizawa, I., Wu, L., Singh, N., Naidenko, O.V., Miura, T., Haba, T., Scherer, D.C., Wei, J., Kronenberg, M., Koezuka, Y., Van Kaer, L., 2001. The natural killer T-cell ligand α-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. Nat. Med. 7, 1052–1056. http://dx.doi.org/10.1038/nm0901-1052.
Ito, S., Iwaki, S., Kondo, R., Satoh, M., Iwabuchi, K., Ohkawa, R., Mishima, Y., Yatomi, Y., Furumoto, T., Tsutsui, H., Fujii, S., To, C., 2014. TNF-a production in NKT cell hybridoma is regulated by sphingosine-1-phosphate: implications for inflammation in atherosclerosis. Coron. Artery Dis. 25, 311–320. http://dx.doi.org/10.1097/MCA.0000000000000082.
Jahng, A., Maricic, I., Aguilera, C., Cardell, S., Halder, R.C., Kumar, V., 2004. Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. J. Exp. Med 199, 947–957. http://dx.doi.org/10.1084/jem.20031389.
Jahng, A.W., Maricic, I., Pedersen, B., Burdin, N., Naidenko, O., Kronenberg, M., Koezuka, Y., Kumar, V., 2001. Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. J. Exp. Med. 194, 1789–1799.
Joyce, S., Woods, A.S., Yewdell, J.W., Bennink, J.R., De Silva, A.D., Boesteanu, A., Balk, S.P., Cotter, R.J., Brutkiewicz, R.R., 1998. Natural ligand of mouse CD1d1: cellular glycosylphosphatidylin. Science 80 (279), 1541–1544.
Kain, L., Webb, B., Anderson, B.L., Deng, S., Holt, M., Costanzo, A., Zhao, M., Self, K., Teyton, A., Everett, C., Kronenberg, M., Zajonc, D.M., Bendelac, A., Savage, P.B., Teyton, L., 2014. The identification of the endogenous ligands of natural killer T cells reveals the presence of mammalian α-linked glycosylceramides. Immunity 41, 543–554. http://dx.doi.org/10.1016/j.immuni.2014.08.017.
Kanellakis, P., Agrotis, A., Kyaw, T.S., Koulis, C., Ahrens, I., Mori, S., Takahashi, H.K., Liu, K., Peter, K., Nishibori, M., Bobik, A., 2011. High-mobility group box protein 1 neutralization reduces development of diet-induced atherosclerosis in apolipoprotein E–deficient mice. Arterioscler. Thromb. Vasc. Biol. 31, 313–319.
Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., Koseki, H., Taniguchi, M., 1997. CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science 80 (278), 1626–1629.
Kee, S.-J., Kwon, Y.-S., Park, Y.-W., Cho, Y.-N., Lee, S.-J., Kim, T.-J., Lee, S.-S., Jang, H.-C., Shin, M.-G., Shin, J.-H., Suh, S.-P., Ryang, D.-W., 2012. Dysfunction of natural killer T cells in patients with active Mycobacterium tuberculosis infection. Infect. Immun. 80, 2100–2108. http://dx.doi.org/10.1128/IAI.06018-11.
Khoo, J.C., Miller, E., McLoughlin, P., Steinberg, D., 1988. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. Arterioscler. Thromb. Vasc. Biol. 8, 348–358.
Khoo, J.C., Miller, E., Pio, F., Steinberg, D., Witztum, J.L., 1992. Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. Arterioscler. Thromb. Vasc. Biol. 12, 1258–1266.
Kinjo, Y., Tupin, E., Wu, D., Fujio, M., Garcia-Navarro, R., Benhnia, M.R.-E.-I., Zajonc, D.M., Ben-Menachem, G., Ainge, G.D., Painter, G.F., Khurana, A., Hoebe, K., Behar, S.M., Beutler, B., Wilson, I.A., Tsuji, M., Sellati, T.J., Wong, C.-H., Kronenberg, M., 2006. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat. Immunol. 7, 978–986. http://dx.doi.org/10.1038/ni1380.
Kis, J., Engelmann, P., Farkas, K., Richman, G., Eck, S., Lolley, J., Jalahej, H., Borowiec, M., Kent, S.C., Treszl, A., Orban, T., 2007. Reduced CD4+ subset and Th1 bias of the human iNKT cells in Type 1 diabetes mellitus. J. Leukoc. Biol. 81, 654–662. http://dx.doi.org/10.1189/jlb.1106654.
Kitamura, H., Iwakabe, K., Yahata, T., Nishimura, S.-I., Ohta, A., Ohmi, Y., Sato, M., Takeda, K., Okumura, K., Kaer, L., Van, Kawano, T., Taniguchi, M., Nishimura, T., 1999. The natural killer T (NKT) cell ligand α-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)–12 production by dendritic cells and IL-12 receptor expression on NKT cells. J. Exp. Med 189, 1121–1127.
Kitamura, H., Ohta, A., Sekimoto, M., Sato, M., Iwakabe, K., Nakui, M., Yahata, T., Meng, H., Koda, T., Nishimura, S., Kawano, T., Taniguchi, M., Nishimura, T., 2000. α-Galactosylceramide induces early B-cell activation through IL-4 production by NKT cells. Cell. Immunol. 199, 37–42. http://dx.doi.org/10.1006/cimm.1999.1602.
Kuboki, S., Sakai, N., Tschöp, J., Edwards, M.J., Lentsch, A.B., Caldwell, C.C., 2009. Distinct contributions of CD4+ T cell subsets in hepatic ischemia/reperfusion injury. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G1054–G1059.
Kyaw, T., Winship, A., Tay, C., Kanellakis, P., Hosseini, H., Cao, A., Li, P., Tipping, P., Bobik, A., Toh, B.-H., 2013. Cytotoxic and proinflammatory CD8+ T lymphocytes promote development of vulnerable atherosclerotic plaques in ApoE-deficient miceclinical perspective. Circulation 127, 1028–1039.
Kyriakakis, E., Cavallari, M., Andert, J., Philippova, M., Koella, C., Bochkov, V., Erne, P., Brian Wilson, S., Mori, L., Biedermann, B.C., Resink, T.J., De Libero, G., 2010. Invariant natural killer T cells: linking inflammation and neovascularization in human atherosclerosis. Eur. J. Immunol. 40, 3268–3279. http://dx.doi.org/10.1002/eji.201040619.
Lappas, C.M., Day, Y.-J., Marshall, M.A., Engelhard, V.H., Linden, J., 2006. Adenosine A2A receptor activation reduces hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation. J. Exp. Med. 203, 2639–2648.
Leite-De-Moraes, M.C., Hameg, A., Arnould, A., Machavoine, F., Koezuka, Y., Schneider, E., Herbelin, A., Dy, M., 1999. A distinct IL-18-induced pathway to fully activate NK T lymphocytes independently from TCR engagement. J. Immunol. 163, 5871–5876.
Leslie, D.S., Dascher, C.C., Cembrola, K., Townes, M.A., Hava, D.L., Hugendubler, L.C., Mueller, E., Fox, L., Roura-Mir, C., Moody, D.B., Vincent, M.S., Gumperz, J.E., Illarionov, P.A., Besra, G.S., Reynolds, C.G., Brenner, M.B., 2008. Serum lipids regulate dendritic cell CD1 expression and function. Immunology 125, 289–301. http://dx.doi.org/10.1111/j.1365-2567.2008.02842.x.
Li, L., Huang, L., Sung, S.J., Lobo, P.I., Brown, M.G., Gregg, R.K., Engelhard, V.H., Okusa, M.D., 2007. NKT cell activation mediates neutrophil IFN-gamma production and renal ischemia-reperfusion injury. J. Immunol. 178, 5899–5911. http://dx.doi.org/10.4049/JIMMUNOL.178.9.5899.
Li, L., Huang, L., Vergis, A.L., Ye, H., Bajwa, A., Narayan, V., Strieter, R.M., Rosin, D.L., Okusa, M.D., 2010. IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J. Clin. Investig. 120, 331–342. http://dx.doi.org/10.1172/JCI38702.
Li, Y., Kanellakis, P., Hosseini, H., Cao, A., Deswaerte, V., Tipping, P., Toh, B.H., Bobik, A., Kyaw, T., 2016. A CD1d-dependent lipid antagonist to NKT cells ameliorates atherosclerosis in ApoE-/- mice by reducing lesion necrosis and inflammation. Cardiovasc. Res. 109, 305–317. http://dx.doi.org/10.1093/cvr/cvv259.
Li, Y., To, K., Kanellakis, P., Hosseini, H., Deswaerte, V., Tipping, P., Smyth, M.J., Toh, B.-H., Bobik, A., Kyaw, T., 2015. CD4+ Natural killer T cells potently augment aortic root atherosclerosis by perforin-and granzyme B- dependent cytotoxicity. Circ. Res. 116, 245–254. http://dx.doi.org/10.1161/CIRCRESAHA.116.304734.
Liu, L.-L., Lǔ, J.-L., Chao, P.-L., Lin, L.-R., Zhang, Z.-Y., Yang, T.-C., 2011. Lower prevalence of circulating invariant natural killer T (iNKT) cells in patients with acute myocardial infarction undergoing primary coronary stenting. Int. Immunopharmacol. 11, 480–484. http://dx.doi.org/10.1016/j.intimp.2010.12.019.
Lombardi, V., Stock, P., Singh, A.K., Kerzerho, J., Yang, W., Sullivan, B.A., Li, X., Shiratsuchi, T., Hnatiuk, N.E., Howell, A.R., Yu, K.O.A., Porcelli, S.A., Tsuji, M., Kronenberg, M., Wilson, S.B., Akbari, O., 2010. A CD1d-dependent antagonist inhibits the activation of invariant NKT cells and prevents development of allergen-induced airway hyperreactivity. J. Immunol. 184, 2107–2115. http://dx.doi.org/10.4049/jimmunol.0901208.
Long, X., Deng, S., Mattner, J., Zang, Z., Zhou, D., McNary, N., Goff, R.D., Teyton, L., Bendelac, A., Savage, P.B., 2007. Synthesis and evaluation of stimulatory properties of Sphingomonadaceae glycolipids. Nat. Chem. Biol. 3, 559–564. http://dx.doi.org/10.1038/nchembio.2007.19.
Major, A.S., Wilson, M.T., McCaleb, J.L., Ru Su, Y., Stanic, A.K., Joyce, S., Van Kaer, L., Fazio, S., Linton, M.F., 2004. Quantitative and qualitative differences in proatherogenic NKT cells in apolipoprotein E–deficient mice. Arterioscler. Thromb. Vasc. Biol. 24, 2351–2357. http://dx.doi.org/10.1161/01.ATV.0000147112.84168.87.
Mattner, J., DeBord, K.L., Ismail, N., Goff, R.D., Cantu, C., Zhou, D., Saint-Mezard, P., Wang, V., Gao, Y., Yin, N., Hoebe, K., Schneewind, O., Walker, D., Beutler, B., Teyton, L., Savage, P.B., Bendelac, A., 2005. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature 434, 525–529. http://dx.doi.org/10.1038/nature03408.
Meliá, A., Geng, Y.-J., Sukhova, G.K., Libby, P., Porcelli, S.A., 1999. CD1 expression in human atherosclerosis a potential mechanism for T cell activation by foam cells. Am. J. Pathol. 155, 775–786. http://dx.doi.org/10.1016/S0002-9440(10)65176-0.
Mera, Y., Kawai, T., Ogawa, N., Odani, N., Sasase, T., Miyajima, K., Ohta, T., Kakutani, M., 2015. JTT-130, a novel intestine-specific inhibitor of microsomal triglyceride transfer protein, ameliorates lipid metabolism and attenuates atherosclerosis in hyperlipidemic animal models. J. Pharmacol. Sci. 129, 169–176. http://dx.doi.org/10.1016/j.jphs.2015.10.004.
Metelitsa, L., Weinberg, K., Emanuel, P., Seeger, R., 2003. Expression of CD1d by myelomonocytic leukemias provides a target for cytotoxic NKT cells. Leukemia 17, 1068–1077. http://dx.doi.org/10.1038/sj.leu.2402943.
Miyamoto, K., Miyake, S., Yamamura, T., 2001. A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. Nature 413, 531–534. http://dx.doi.org/10.1038/35097097.
Mizuno, M., Masumura, M., Tomi, C., Chiba, A., Oki, S., Yamamura, T., Miyake, S., 2004. Synthetic glycolipid OCH prevents insulitis and diabetes in NOD mice. J. Autoimmun. 23, 293–300. http://dx.doi.org/10.1016/j.jaut.2004.09.008.
Monzon-Casanova, E., Steiniger, B., Schweigle, S., Clemen, H., Zdzieblo, D., Starick, L., Müller, I., Wang, C.-R., Rhost, S., Cardell, S., Pyz, E., Herrmann, T., 2010. CD1d expression in paneth cells and rat exocrine pancreas revealed by novel monoclonal antibodies which differentially affect NKT cell activation. PLoS One, 5. http://dx.doi.org/10.1371/journal.pone.0013089.
Morita, M., Motoki, K., Akimoto, K., Natori, T., Sakai, T., Sawa, E., Yamaji, K., Koezuka, Y., Kobayashi, E., Fukushima, H., 1995. Structure-activity relationship of.alpha.-Galactosylceramides against B16-bearing mice. J. Med. Chem. 38, 2176–2187. http://dx.doi.org/10.1021/jm00012a018.
Mukhin, D.N., Chao, F.-F., Kruth, H.S., 1995. Glycosphingolipid accumulation in the aortic wall is another feature of human atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 15, 1607–1615.
Mureithi, M.W., Cohen, K., Moodley, R., Poole, D., Mncube, Z., Kasmar, A., Moody, D.B., Goulder, P.J.R., Walker, B.D., Altfeld, M., Ndung'u, T., 2011. Impairment of CD1d-restricted natural killer T cells in chronic HIV type 1 clade C infection. AIDS Res. Hum. Retrovir. 27, 501–509. http://dx.doi.org/10.1089/AID.2010.0237.
Nagaraj, S., Ziske, C., Strehl, J., Messmer, D., Sauerbruch, T., Schmidt-Wolf, I.G.H., 2006. Dendritic cells pulsed with alpha-galactosylceramide induce anti-tumor immunity against pancreatic cancer in vivo. Int. Immunol. 18, 1279–1283. http://dx.doi.org/10.1093/intimm/dxl059.
Nakai, Y., Iwabuchi, K., Fujii, S., Ishimori, N., Dashtsoodol, N., Watano, K., Mishima, T., Iwabuchi, C., Tanaka, S., Bezbradica, J.S., Nakayama, T., Taniguchi, M., Miyake, S., Yamamura, T., Kitabatake, A., Joyce, S., Kaer, L., Van, Onoé, K., 2004. Natural killer T cells accelerate atherogenesis in mice. Blood 104, 2051–2059. http://dx.doi.org/10.1182/blood-2003-10-3485.
Nakamura, T., Kuroi, M., Harashima, H., 2015. Influence of endosomal escape and degradation of α-galactosylceramide loaded liposomes on CD1d antigen presentation. Mol. Pharm. 12, 2791–2799. http://dx.doi.org/10.1021/mp500704e.
Nakamura, T., Yamazaki, D., Yamauchi, J., Harashima, H., 2013. The nanoparticulation by octaarginine-modified liposome improves α-galactosylceramide-mediated antitumor therapy via systemic administration. J. Control. Release 171, 216–224. http://dx.doi.org/10.1016/j.jconrel.2013.07.004.
Nishimura, T., Kitamura, H., Iwakabe, K., Yahata, T., Ohta, A., Sato, M., Takeda, K., Okumura, K., Van Kaer, L., Kawano, T., Taniguchi, M., Nakui, M., Sekimoto, M., Koda, T., 2000. The interface between innate and acquired immunity: glycolipid antigen presentation by CD1d-expressing dendritic cells to NKT cells induces the differentiation of antigen-specific cytotoxic T lymphocytes. Int. Immunol. 12, 987–994. http://dx.doi.org/10.1093/INTIMM/12.7.987.
Novak, J., Dobrovolny, J., Tousek, P., Kocka, V., Teringova, E., Novakova, L., Widimsky,

P., 2015. Potential role of invariant natural killer T cells in outcomes of acute myocardial infarction. Int. J. Cardiol. 187, 663–665. http://dx.doi.org/10.1016/j.ijcard.2015.03.398.

Ohmura, K., Ishimori, N., Ohmura, Y., Tokuhara, S., Nozawa, A., Horii, S., Andoh, Y., Fujii, S., Iwabuchi, K., Onoé, K., Tsutsui, H., 2009. Natural killer T cells are involved in adipose tissues inflammation and glucose intolerance in diet-induced obese mice. Arterioscler. Thromb. Vasc. Biol. 30, 193–199. http://dx.doi.org/10.1161/ATVBAHA.109.198614.

Oki, S., Chiba, A., Yamamura, T., Miyake, S., 2004. The clinical implication and molecular mechanism of preferential IL-4 production by modified glycolipid-stimulated NKT cells. J. Clin. Investig. 113, 1631–1640. http://dx.doi.org/10.1172/JCI20862.

Ostos, M.A., Recalde, D., Zakin, M.M., Scott-Algara, D., 2002. Implication of natural killer T cells in atherosclerosis development during a LPS-induced chronic inflammation. FEBS Lett. 519, 23–29. http://dx.doi.org/10.1016/S0014-5793(02)02692-3.

Rogers, L., Burchat, S., Gage, J., Hasu, M., Thabet, M., Wilcox, L., Ramsamy, T.A., Whitman, S.C., 2008. Deficiency of invariant Va14 natural killer T cells decreases atherosclerosis in LDL receptor null mice. Cardiovasc. Res. 78, 167–174. http://dx.doi.org/10.1093/cvr/cvn005.

Rombouts, M., Ammi, R., Van Brussel, I., Roth, L., De Winter, B.Y., Vercauteren, S.R., Hendriks, J.M.H., Lauwers, P., Van Schil, P.E., De Meyer, G.R.Y., Fransen, E., Cools, N., Schrijvers, D.M., 2016. Linking CD11b (+) dendritic cells and natural killer T cells to plaque inflammation in atherosclerosis. Mediat. Inflamm. 2016, 6467375. http://dx.doi.org/10.1155/2016/6467375.

Saubermann, L.J., Beck, P., De Jong, Y.P., Pitman, R.S., Ryan, M.S., Kim, H.S., Snapper, S., Hagen, S.J., Kanauchi, O., Motoki, K., Sakai, T., Terhorst, C., Koezuka, Y., Podolsky, D.K., Blumberg, R.S., Exley, M., Balk, S.P., 2000. Activation of natural killer T cells by α-galactosylceramide in the presence of CD1d provides protection against colitis in mice. Gastroenterology 119, 119–128. http://dx.doi.org/10.1053/gast.2000.9114.

Shimamura, K., Kawamura, H., Nagura, T., Kato, T., Naito, T., Kameyama, H., Hatakeyama, K., Abo, T., 2005. Association of NKT cells and granulocytes with liver injury after reperfusion of the portal vein. Cell. Immunol. 234, 31–38. http://dx.doi.org/10.1016/j.cellimm.2005.04.022.

Sobirin, M.A., Kinugawa, S., Takahashi, M., Fukushima, A., Homma, T., Ono, T., Hirabayashi, K., Suga, T., Azalia, P., Takada, S., Taniguchi, M., Nakayama, T., Ishimori, N., Iwabuchi, K., Tsutsui, H., 2012. Activation of natural killer T cells ameliorates postinfarct cardiac remodeling and failure in micenovelty and significance. Circ. Res. 111, 1037–1047.

Soh, S.Y., Faveeuw, C., Thiam, C.H., Khoo, L.H.B., Yeo, K.P., Lim, S.Y., Lim, H.Y., Ng, J.X., Angeli, V., 2016. NKT cell hyporesponsiveness leads to unrestrained accumulation of marginal zone B cells in hypercholesterolemic apolipoprotein E–deficient mice. J. Immunol. 197, 3894–3904. http://dx.doi.org/10.4049/jimmunol.1500999.

Stemme, S., Faber, B., Holm, J., Wiklund, O., Witztum, J.L., Hansson, G.K., 1995. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc. Natl. Acad. Sci. USA 92, 3893–3897.

Ström, Å., Wigren, M., Hultgårdh-Nilsson, A., Saxena, A., Gomez, M.F., Cardell, S., Fredrikson, G.N., Nilsson, J., 2007. Involvement of the CD1d–natural killer T cell pathway in neointima formation after vascular injury. Circ. Res. 101, e83–e89. http://dx.doi.org/10.1161/CIRCRESAHA.107.160705.

Subramanian, S., Turner, M.S., Ding, Y., Goodspeed, L., Wang, S., Buckner, J.H., O'Brien, K., Getz, G.S., Reardon, C.A., Chait, A., 2013. Increased levels of invariant natural killer T lymphocytes worsen metabolic abnormalities and atherosclerosis in obese mice. J. Lipid Res. 54, 2831–2841. http://dx.doi.org/10.1194/jlr.M041020.

Szatmari, I., Pap, A., Rühl, R., Ma, J.-X., Illarionov, P.A., Besra, G.S., Rajnavolgyi, E., Dezso, B., Nagy, L., 2006. PPARgamma controls CD1d expression by turning on retinoic acid synthesis in developing human dendritic cells. J. Exp. Med. 203, 2351–2362. http://dx.doi.org/10.1084/jem.20060141.

Tahir, S.M., Cheng, O., Shaulov, A., Koezuka, Y., Bubley, G.J., Wilson, S.B., Balk, S.P., Exley, M.A., 2001. Loss of IFN-gamma production by invariant NK T cells in advanced cancer. J. Immunol. 167, 4046–4050. http://dx.doi.org/10.4049/JIMMUNOL.167.7.4046.

Thomas, S.Y., Hou, R., Boyson, J.E., Means, T.K., Hess, C., Olson, D.P., Strominger, J.L., Brenner, M.B., Gumperz, J.E., Wilson, S.B., Luster, A.D., 2003. CD1d-restricted NKT cells express a chemokine receptor profile indicative of Th1-type inflammatory homing cells. J. Immunol. 171, 2571–2580. http://dx.doi.org/10.4049/JIMMUNOL.171.5.2571.

To, K., Agrotis, A., Besra, G., Bobik, A., Toh, B.-H., 2009. NKT cell subsets mediate differential proatherogenic effects in ApoE−/− mice. Arterioscler. Thromb. Vasc. Biol. 29, 671–677.

Tsuji, M., 2006. Glycolipids and phospholipids as natural CD1d-binding NKT cell ligands. Cell. Mol. Life Sci. 63, 1889–1898. http://dx.doi.org/10.1007/s00018-006-6073-z.

Tsutahara, K., Okumi, M., Kakuta, Y., Abe, T., Yazawa, K., Miyagawa, S., Matsunami, K., Otsuka, H., Kaimori, J., Takahara, S., Nonomura, N., 2012. The blocking of CXCR3 and CCR5 suppresses the infiltration of T lymphocytes in rat renal ischemia reperfusion. Nephrol. Dial. Transplant. 27, 3799–3806. http://dx.doi.org/10.1093/ndt/gfs360.

Tupin, E., Nicoletti, A., Elhage, R., Rudling, M., Ljunggren, H.-G., Hansson, G.K., Paulsson Berne, G., 2004. CD1d-dependent activation of NKT cells aggravates atherosclerosis. J. Exp. Med 199, 417–422. http://dx.doi.org/10.1084/jem.20030997.

Ueno, Y., Tanaka, S., Sumii, M., Miyake, S., Tazuma, S., Taniguchi, M., Yamamura, T., Chayama, K., 2005. Single dose of OOCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of Vα14 natural killer T cells in mice. Inflamm. Bowel Dis. 11, 35–41. http://dx.doi.org/10.1097/00054725-200501000-00005.

van der Vliet, H.J., von Blomberg, B.M., Nishi, N., Reijm, M., Voskuyl, A.E., van Bodegraven, A.A., Polman, C.H., Rustemeyer, T., Lips, P., van den Eertwegh, A.J., Giaccone, G., Scheper, R.J., Pinedo, H.M., 2001. Circulating V(alpha24+) Vbeta11+ NKT cell numbers are decreased in a wide variety of diseases that are characterized by autoreactive tissue damage. Clin. Immunol. 100, 144–148. http://dx.doi.org/10.1006/clim.2001.5060.

Van Kaer, L., Parekh, V.V., Wu, L., 2011. Invariant NK T cells: potential for immunotherapeutic targeting with glycolipid antigens. Immunotherapy 3, 59–75. http://dx.doi.org/10.2217/imt.10.85.

van Puijvelde, G.H., van Wanrooij, E.J., Hauer, A.D., de Vos, P., van Berkel, T.J., Kuiper, J., To, C., Kuiper, J., 2009. Effect of natural killer T cell activation on initiation of atherosclerosis Theme Issue Article. Thromb. Haemost. 102, 223–230. http://dx.doi.org/10.1160/TH09-01-0020.

VanderLaan, P.A., Reardon, C.A., Sagiv, Y., Blachowicz, L., Lukens, J., Nissenbaum, M., Wang, C.-R., Getz, G.S., 2007. Characterization of the natural killer T-cell response in an adoptive transfer model of atherosclerosis. Am. J. Pathol. 170, 1100–1107. http://dx.doi.org/10.2353/ajpath.2007.060188.

Villanueva, A.I., Haeryfar, S.M.M., Mallard, B.A., Kulkarni, R.R., Sharif, S., 2015. Functions of invariant NK T cells are modulated by TLR ligands and IFN-α. Innate Immun. 21, 275–288. http://dx.doi.org/10.1177/1753425914527327.

Wong, C.H.Y., Jenne, C.N., Lee, W.-Y., Léger, C., Kubes, P., 2011. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. Science 80 (334), 101–105.

Wu, D.Y., Segal, N.H., Sidobre, S., Kronenberg, M., Chapman, P.B., 2003. Cross-presentation of disialoganglioside GD3 to natural killer T cells. J. Exp. Med. 198, 173–181. http://dx.doi.org/10.1084/jem.20030446.

Yan, X., Anzai, A., Katsumata, Y., Matsuhashi, T., Ito, K., Endo, J., Yamamoto, T., Takeshima, A., Shinmura, K., Shen, W., Fukuda, K., Sano, M., 2013. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. J. Mol. Cell. Cardiol. 62, 24–35. http://dx.doi.org/10.1016/j.yjmcc.2013.04.023.

Yanagihara, Y., Shiozawa, K., Takai, M., Kyogoku, M., Shiozawa, S., 1999. Natural killer (NK) T cells are significantly decreased in the peripheral blood of patients with rheumatoid arthritis (RA). Clin. Exp. Immunol. 118, 131–136. http://dx.doi.org/10.1046/j.1365-2249.1999.01018.x.

Yanai, H., Yoshida, H., Tomono, Y., Tada, N., Chiba, H., 2006. The possible contribution of a general glycosphingolipid transporter, GM2 activator protein, to atherosclerosis. J. Atheroscler. Thromb. 13, 281–285. http://dx.doi.org/10.5551/jat.13.281.

Yang, G., Schmieg, J., Tsuji, M., Franck, R.W., 2004. The C-glycoside analogue of the immunostimulantα-galactosylceramide (KRN7000): synthesis and striking enhancement of activity. Angew. Chem. Int. Ed. 43, 3818–3822. http://dx.doi.org/10.1002/anie.200454215.

Yang, S.H., Lee, J.P., Jang, H.R., Cha, R., Han, S.S., Jeon, U.S., Kim, D.K., Song, J., Lee, D.-S., Kim, Y.S., 2011. Sulfatide-reactive natural killer T cells abrogate ischemia-reperfusion injury. J. Am. Soc. Nephrol. 22, 1305–1314. http://dx.doi.org/10.1681/ASN.2010080815.

Zajonc, D.M., Ainge, G.D., Painter, G.F., Severn, W.B., Wilson, I.A., 2006. Structural characterization of mycobacterial phosphatidylinositol mannoside binding to mouse CD1d. J. Immunol. 177, 4577–4583. http://dx.doi.org/10.4049/JIMMUNOL.177.7.4577.

Zhang, C., Zheng, L., Li, L., Wang, L., Li, L., Huang, S., Gu, C., Zhang, L., Yang, C., Zhu, T., Rong, R., 2014. Rapamycin protects kidney against ischemia reperfusion injury through recruitment of NKT cells. J. Transl. Med. 12, 224. http://dx.doi.org/10.1186/s12967-014-0224-z.

Zhang, J., Han, C., Dai, H., Hou, J., Dong, Y., Cui, X., Xu, L., Zhang, M., Xia, Q., 2016. Hypoxia-inducible factor-2α limits natural killer T cell cytotoxicity in renal ischemia/reperfusion injury. J. Am. Soc. Nephrol. 27, 92–106. http://dx.doi.org/10.1681/ASN.2014121248.

Zhou, D., Cantu, C., Sagiv, Y., Schrantz, N., Kulkarni, A.B., Qi, X., Mahuran, D.J., Morales, C.R., Grabowski, G.A., Benlagha, K., Savage, P., Bendelac, A., Teyton, L., Teyton, L., 2004a. Editing of CD1d-bound lipid antigens by endosomal lipid transfer proteins. Science 303, 523–527. http://dx.doi.org/10.1126/science.1092009.

Zhou, D., Mattner, J., Cantu, C., Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K., Wu, Y.-P., Yamashita, T., Teneberg, S., Wang, D., Proia, R.L., Levery, S.B., Savage, P.B., Teyton, L., Bendelac, A., 2004b. Lysosomal glycosphingolipid recognition by NKT cells. Science 80 (306), 1786–1789.